

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 October 2001 (11.10.2001)

PCT

(10) International Publication Number
WO 01/74765 A1

(51) International Patent Classification⁷: C07C 401/00, A61K 31/59

(21) International Application Number: PCT/US01/10094

(22) International Filing Date: 29 March 2001 (29.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/541,470 31 March 2000 (31.03.2000) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

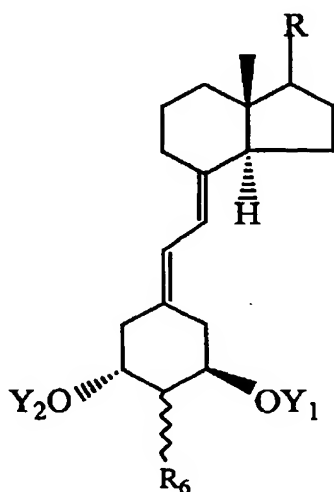
(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: 26,27-HOMOLOGATED-20-EPI-2-ALKYL-19-NOR-VITAMIN D COMPOUNDS



(I)

(57) Abstract: This invention provides 2-alkyl-19-nor-vitamin D derivatives, as well as a general method for their chemical synthesis. The compounds have formula (I) where Y₁ and Y₂, which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, R₆ is selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, and where the group R represents any of the typical side chains known for vitamin D type compounds. These compounds are characterized by relatively high intestinal calcium transport activity and relatively high bone calcium mobilization activity resulting in novel therapeutic agents for the treatment of diseases where bone formation is desired, particularly low bone turnover osteoporosis. These compounds also exhibit pronounced activity in arresting the proliferation of undifferentiated cells and inducing their differentiation to the monocyte thus evidencing use as anti-cancer agents and for the treatment of diseases such as psoriasis.

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26,27-HOMOLOGATED-20-EPI-
2-ALKYL-19-NOR-VITAMIN D COMPOUNDS

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BACKGROUND OF THE INVENTION

This patent invention relates to vitamin D compounds, and more particularly to vitamin D derivatives substituted at the carbon 2 position.

10 The natural hormone, $1\alpha,25$ -dihydroxyvitamin D_3 and its analog in ergosterol series, i.e. $1\alpha,25$ -dihydroxyvitamin D_2 are known to be highly potent regulators of calcium homeostasis in animals and humans, and more recently their activity in cellular differentiation has been established, Ostrem et al., Proc. Natl. Acad. Sci. USA, 84, 2610 (1987). Many structural analogs of these metabolites have been prepared and tested,
15 including 1α -hydroxyvitamin D_3 , 1α -hydroxyvitamin D_2 , various side chain homologated vitamins and fluorinated analogs. Some of these compounds exhibit an interesting separation of activities in cell differentiation and calcium regulation. This difference in activity may be useful in the treatment of a variety of diseases such as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain
20 malignancies.

 Recently, a new class of vitamin D analogs has been discovered, i.e. the so called 19-nor-vitamin D compounds, which are characterized by the replacement of the A-ring exocyclic methylene group (carbon 19), typical of the vitamin D system, by two hydrogen

atoms. Biological testing of such 19-nor-analogs (e.g., 1 α ,25-dihydroxy-19-nor-vitamin D₃) revealed a selective activity profile with high potency in inducing cellular differentiation, and very low calcium mobilizing activity. Thus, these compounds are potentially useful as therapeutic agents for the treatment of malignancies, or the treatment of various skin disorders. Two different methods of synthesis of such 19-nor-vitamin D analogs have been described (Perlman et al., Tetrahedron Lett. 31, 1823 (1990); Perlman et al., Tetrahedron Lett. 32, 7663 (1991), and DeLuca et al., U.S. Pat. No. 5,086,191).

In U.S. Pat. No. 4,666,634, 2 β -hydroxy and alkoxy (e.g., ED-71) analogs of 1 α ,25-dihydroxyvitamin D₃ have been described and examined by Chugai group as potential drugs for osteoporosis and as antitumor agents. See also Okano et al., Biochem. Biophys. Res. Commun. 163, 1444 (1989). Other 2-substituted (with hydroxyalkyl, e.g., ED-120, and fluoroalkyl groups) A-ring analogs of 1 α ,25-dihydroxyvitamin D₃ have also been prepared and tested (Miyamoto et al., Chem. Pharm. Bull. 41, 1111 (1993); Nishii et al., Osteoporosis Int. Suppl. 1, 190 (1993); Posner et al., J. Org. Chem. 59, 7855 (1994), and J. Org. Chem. 60, 4617 (1995)).

Recently, 2-substituted analogs of 1 α ,25-dihydroxy-19-norvitamin D₃ have also been synthesized, i.e. compounds substituted at 2-position with hydroxy or alkoxy groups (DeLuca et al., U.S. Pat. No. 5,536,713), which exhibit interesting and selective activity profiles. All these studies indicate that binding sites in vitamin D receptors can accommodate different substituents at C-2 in the synthesized vitamin D analogs.

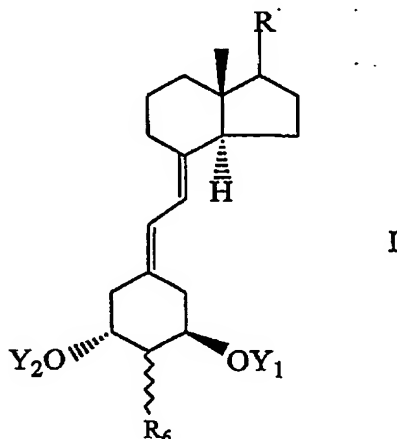
In a continuing effort to explore the 19-nor class of pharmacologically important vitamin D compounds, their analogs which are characterized by the presence of an alkyl (particularly methyl) substituent at the carbon 2 (C-2), i.e. 2-alkyl-19-nor-vitamin D compounds, and particularly 2-methyl-19-nor-vitamin D compounds, have now been synthesized and tested. Such vitamin D analogs seemed interesting targets because the relatively small alkyl (particularly methyl) group at C-2 should not interfere with binding to the vitamin D receptor. On the other hand it is obvious that a change of conformation of the cyclohexanediol ring A can be expected for these new analogs.

BRIEF SUMMARY OF THE INVENTION

A class of 1 α -hydroxylated vitamin D compounds not known heretofore are the 19-nor-vitamin D analogs having an alkyl (particularly methyl) group at the 2-position, i.e.

2-alkyl-19-nor-vitamin D compounds, particularly 2-methyl-19-nor-vitamin D compounds.

Structurally these novel analogs are characterized by the general formula I shown below:

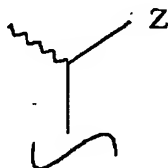


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where Y_1 and Y_2 , which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, R_6 is selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, and where the group R represents any of the typical side chains known for vitamin D type compounds.

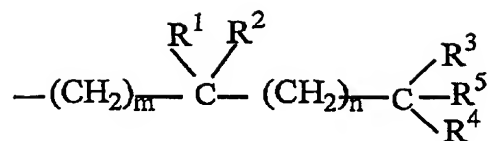
More specifically R can represent a saturated or unsaturated hydrocarbon radical of 1 to 35 carbons, that may be straight-chain, branched or cyclic and that may contain one or more additional substituents, such as hydroxy- or protected-hydroxy groups, fluoro, carbonyl, ester, epoxy, amino or other heteroatomic groups. Preferred side chains of this type are represented by the structure below

15



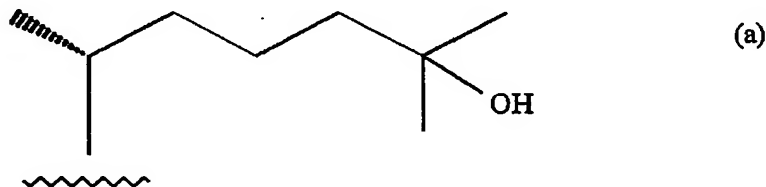
where the stereochemical center (corresponding to C-20 in steroid numbering) may have the R or S configuration, (i.e. either the natural configuration about carbon 20 or the 20-epi configuration), and where Z is selected from Y , $-OY$, $-CH_2OY$, $-C\equiv CY$, $-CH=CHY$, and $-CH_2CH_2CH=CR^3R^4$, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:

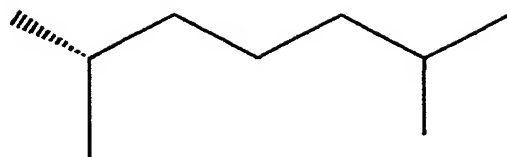
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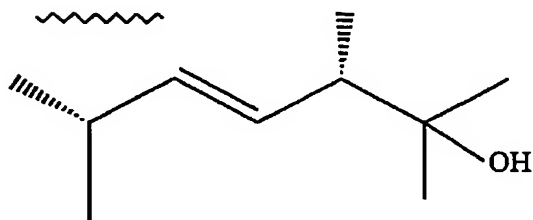
- where m and n, independently, represent the integers from 0 to 5, where R¹ is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C₁₋₅-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R², R³, and R⁴, independently, is selected from deuterium, deutoalkyl, hydrogen, fluoro, trifluoromethyl and C₁₋₅ alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R¹ and R², taken together, represent an oxo group, or an alkylidene group, =CR²R³, or the group -(CH₂)_p-, where p is an integer from 2 to 5, and where R³ and R⁴, taken together, represent an oxo group, or the group -(CH₂)_q-, where q is an integer from 2 to 5, and where R⁵ represents hydrogen, hydroxy, protected hydroxy, C₁₋₅ alkyl or -OR⁷ where R⁷ represents C₁₋₅ alkyl, and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -CH(CH₃)-, -CH(R³)-, or -CH(R²)- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

- The wavy lines to the substituents at C-2 and at C-20 indicate that the carbon 2 and carbon 20 may have either the R or S configuration.
- Specific important examples of side chains with natural 20*R*-configuration are the structures represented by formulas (a), (b), (c), (d) and (e) below. i.e. the side chain as it occurs in 25-hydroxyvitamin D₃ (a); vitamin D₃ (b); 25-hydroxyvitamin D₂ (c); vitamin D₂ (d); and the C-24 epimer of 25-hydroxyvitamin D₂ (e):

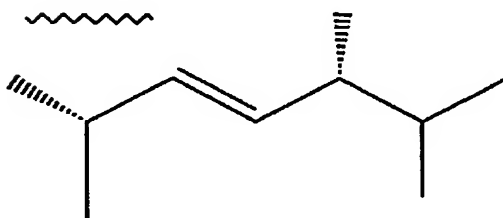




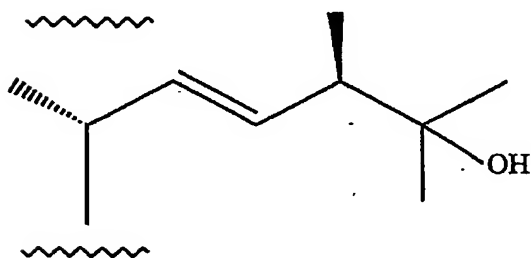
(b)



(c)



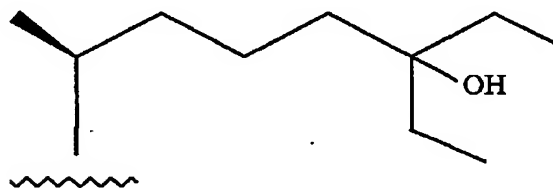
(d)



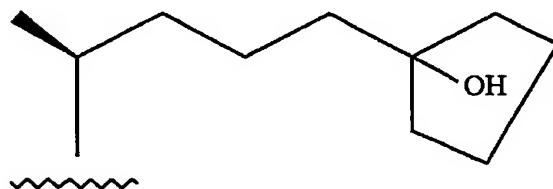
(e)

Specific important examples of side chains with the unnatural 20S (also referred to as the 20-epi) configuration are the structures presented by formulas (f) and (g) below:

5



(f)



(g)

The above novel compounds exhibit a desired, and highly advantageous, pattern of biological activity. These compounds are characterized by relatively high intestinal calcium transport activity, as compared to that of $1\alpha,25$ -dihydroxyvitamin D_3 , while also exhibiting relatively high activity, as compared to $1\alpha,25$ -dihydroxyvitamin D_3 , in their ability to mobilize calcium from bone. Hence, these compounds are highly specific in their calcemic activity. Their preferential activity on mobilizing calcium from bone and either high or normal intestinal calcium transport activity allows the in vivo administration of these compounds for the treatment of metabolic bone diseases where bone loss is a major concern. Because of their preferential calcemic activity on bone, these compounds would be preferred therapeutic agents for the treatment of diseases where bone formation is desired, such as osteoporosis, especially low bone turnover osteoporosis, steroid induced osteoporosis, senile osteoporosis or postmenopausal osteoporosis, as well as osteomalacia and renal osteodystrophy. The treatment may be transdermal, oral or parenteral. The compounds may be present in a composition in an amount from about $0.1\mu\text{g/gm}$ to about $50\mu\text{g/gm}$ of the composition, and may be administered in dosages of from about $0.01\mu\text{g/day}$ to about $50\mu\text{g/day}$.

The compounds of the invention are also especially suited for treatment and prophylaxis of human disorders which are characterized by an imbalance in the immune system, e.g. in autoimmune diseases, including multiple sclerosis, diabetes mellitus, host versus graft reaction, and rejection of transplants; and additionally for the treatment of inflammatory diseases, such as rheumatoid arthritis and asthma, as well as the improvement of bone fracture healing and improved bone grafts. Acne, alopecia, skin conditions such as dry skin (lack of dermal hydration), undue skin slackness (insufficient skin firmness), insufficient sebum secretion and wrinkles, and hypertension are other conditions which may be treated with the compounds of the invention.

The above compounds are also characterized by high cell differentiation activity. Thus, these compounds also provide therapeutic agents for the treatment of psoriasis, or as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer and prostate cancer. The compounds may be present in a composition to treat psoriasis in an amount from about $0.01\mu\text{g/gm}$ to about $100\mu\text{g/gm}$ of the composition, and may be administered

topically, transdermally, orally or parenterally in dosages of from about 0.01 μ g/day to about 100 μ g/day.

This invention also provides novel intermediate compounds formed during the synthesis of the end products.

- 5 This invention also provides a novel synthesis for the production of the end products of structure I

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph illustrating the relative activity of a mixture of 2 α and 2 β -methyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, a mixture of 2 α and 2 β -methyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃ to compete for binding of [³H]-1,25-(OH)₂-D₃ to the pig intestinal nuclear vitamin D receptor;

Figure 2 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of a mixture of 2 α and 2 β -methyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, a mixture of 2 α and 2 β -methyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃;

Figure 3 is a graph similar to Fig. 1 except illustrating the relative activity of the individual compounds 2 α and 2 β -methyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, 2 α and 2 β -methyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃ to compete for binding of [³H]-1,25-(OH)₂-D₃ to the vitamin D pig intestinal nuclear receptor;

Figure 4 is a graph similar to Fig. 2 except illustrating the percent HL-60 cell differentiation as a function of the concentration of the individual compounds 2 α and 2 β -methyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, 2 α and 2 β -methyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃;

Fig. 5 is a graph illustrating the relative activity of the individual compounds 2 α and 2 β -hydroxymethyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, 2 α and 2 β -hydroxymethyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃ to compete for binding of [³H]-1,25-(OH)₂-D₃ to the vitamin D pig intestinal nuclear receptor; and

Fig. 6 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of the individual compounds 2 α and 2 β -hydroxymethyl-19-nor-20S-1 α ,25-dihydroxyvitamin D₃, 2 α and 2 β -hydroxymethyl-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃.

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DETAILED DESCRIPTION OF THE INVENTION

As used in the description and in the claims, the term "hydroxy-protecting group" signifies any group commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl or alkylarylsilyl groups (hereinafter referred to simply as "silyl" groups), and alkoxyalkyl groups. Alkoxycarbonyl protecting groups are alkyl-O-CO- groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term "acyl" signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, malonyl, succinyl, glutaryl group, or an aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word "alkyl" as used in the description or the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all its isomeric forms. Alkoxyalkyl protecting groups are groupings such as methoxymethyl, ethoxymethyl, methoxyethoxymethyl, or tetrahydrofuranyl and tetrahydropyranyl. Preferred silyl-protecting groups are trimethylsilyl, triethylsilyl, t-butyl dimethylsilyl, dibutylmethylsilyl, diphenylmethylsilyl, phenyldimethylsilyl, diphenyl-t-butylsilyl and analogous alkylated silyl radicals. The term "aryl" specifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

A "protected hydroxy" group is a hydroxy group derivatised or protected by any of the above groups commonly used for the temporary or permanent protection of hydroxy functions, e.g. the silyl, alkoxyalkyl, acyl or alkoxycarbonyl groups, as previously defined. The terms "hydroxyalkyl", "deuteroalkyl" and "fluoroalkyl" refer to an alkyl radical substituted by one or more hydroxy, deuterium or fluoro groups respectively.

It should be noted in this description that the term "24-homo" refers to the addition of one methylene group and the term "24-dihomo" refers to the addition of two methylene groups at the carbon 24 position in the side chain. Likewise, the term "trihomo" refers to the addition of three methylene groups. Also, the term "26,27-

dimethyl" refers to the addition of a methyl group at the carbon 26 and 27 positions so that for example R³ and R⁴ are ethyl groups. Likewise, the term "26,27-diethyl" refers to the addition of an ethyl group at the 26 and 27 positions so that R³ and R⁴ are propyl groups.

In the following lists of compounds, the particular substituent attached at the carbon 2 position should be added to the nomenclature. For example, if a methyl group is the alkyl substituent, the term "2-methyl" should precede each of the named compounds. If an ethyl group is the alkyl substituent, the term "2-ethyl" should precede each of the named compounds, and so on. In addition, if the methyl group attached at the carbon 20 position is in its epi or unnatural configuration, the term "20(S)" or "20-epi" should be included in each of the following named compounds. Also, if the side chain contains an oxygen atom substituted at any of positions 20, 22 or 23, the term "20-oxa", "22-oxa" or "23-oxa", respectively, should be added to the named compound. The named compounds could also be of the vitamin D₂ type if desired.

Specific and preferred examples of the 2-alkyl-compounds of structure I when the side chain is unsaturated are:

19-nor-24-homo-1,25-dihydroxy-22,23-dehydrovitamin D₃ ;
19-nor-24-dihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-24-trihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-dimethyl-24-homo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-diethyl-24-homo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-dipropoyl-24-homo-1,25-dihydroxy-22,23-dehydrovitamin D₃;
19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃;

and

19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxy-22,23-dehydrovitamin D₃.

With respect to the above unsaturated compounds, it should be noted that the double bond located between the 22 and 23 carbon atoms in the side chain may be in either the (E) or (Z) configuration. Accordingly, depending upon the configuration, the term "22,23(E)" or "22,23(Z)" should be included in each of the above named compounds.

Also, it is common to designate the double bond located between the 22 and 23 carbon atoms with the designation " Δ^{22} ". Thus, for example, the first named compound above could also be written as 19-nor-24-homo-22,23(E)- Δ^{22} -1,25-(OH)₂D₃ where the double bond is in the (E) configuration. Similarly, if the methyl group attached at carbon 20 is in the unnatural configuration, this compound could be written as 19-nor-20(S)-24-homo-22,23(E)- Δ^{22} -1,25-(OH)₂D₃.

Specific and preferred examples of the 2-alkyl-compounds of structure I when the side chain is saturated are:

- 19-nor-24-homo-1,25-dihydroxyvitamin D₃;
- 10 19-nor-24-dihomo-1,25-dihydroxyvitamin D₃;
- 19-nor-24-trihomo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dimethyl-24-homo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxyvitamin D₃;
- 15 19-nor-26,27-diethyl-24-homo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dipropyl-24-homo-1,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxyvitamin D₃; and
- 20 19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxyvitamin D₃.

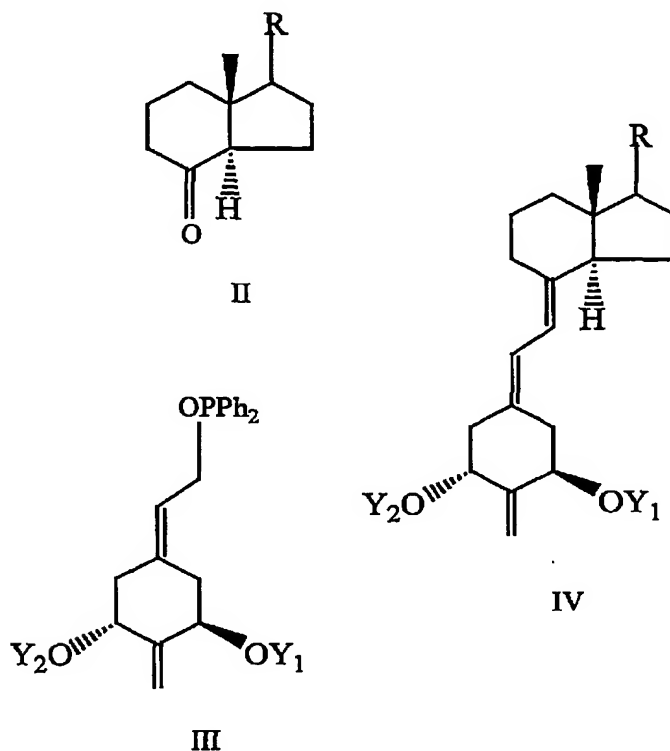
As noted previously, the above saturated side chain compounds should have the appropriate 2-alkyl substituent and/or carbon 20 configuration added to the nomenclature. For example, particularly preferred compounds are:

- 19-nor-26,27-dimethyl-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃; which can
- 25 also be written as 19-nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dimethyl-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃; which can
- also be written as 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃;
- 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃; and
- 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

The preparation of 1 α -hydroxy-2-alkyl-19-nor-vitamin D compounds, particularly 1 α -hydroxy-2-methyl-19-nor-vitamin D compounds, having the basic

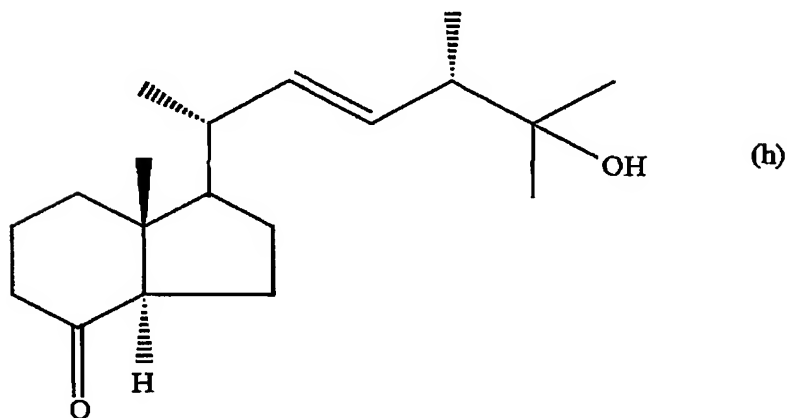
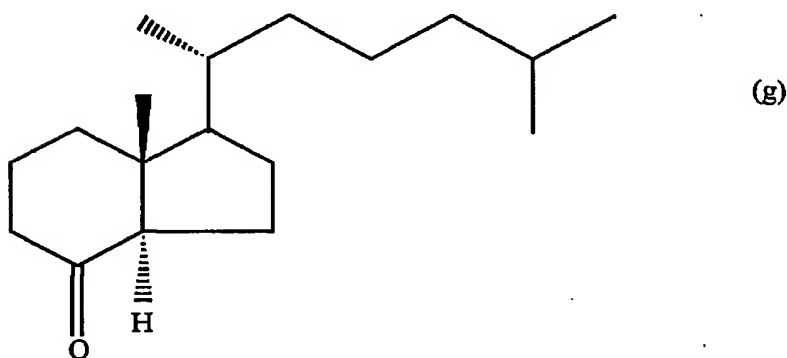
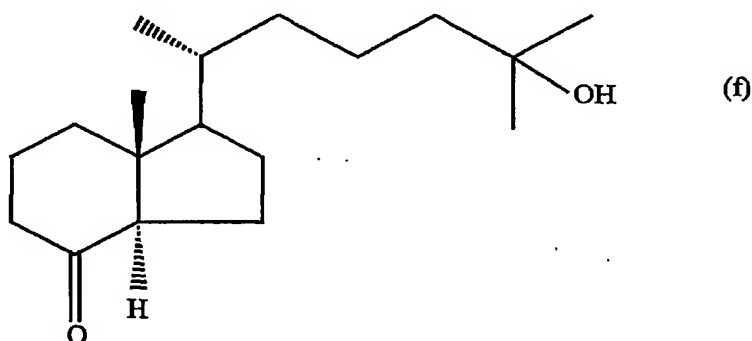
structure I can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone II with the allylic phosphine oxide III to the corresponding 2-methylene-19-nor-vitamin D analogs IV followed by a selective reduction of the exomethylene group at C-2 in the latter compounds:

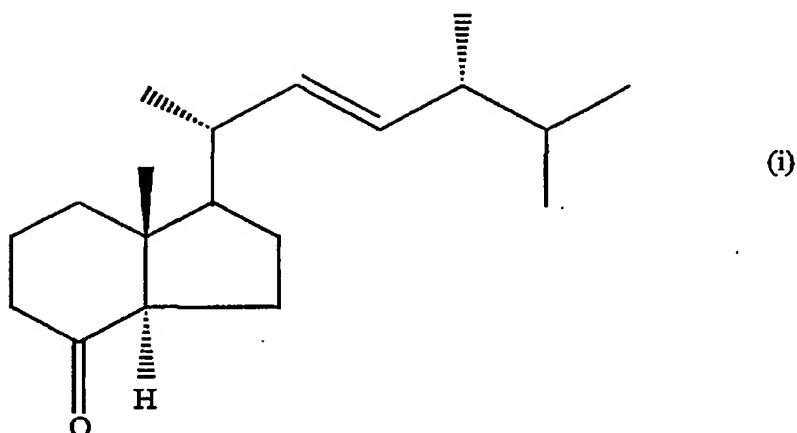
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In the structures II, III, and IV groups Y_1 and Y_2 and R represent groups defined above; Y_1 and Y_2 are preferably hydroxy-protecting groups, it being also understood that any
10 functionalities in R that might be sensitive, or that interfere with the condensation reaction, be suitable protected as is well-known in the art. The process shown above represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds [e.g. Lythgoe et al., J. Chem. Soc. Perkin Trans. I, 590 (1978); Lythgoe, Chem. Soc. Rev. 9, 449 (1983); Toh et al., J. Org. Chem. 48, 1414
15 (1983); Baggiolini et al., J. Org. Chem. 51, 3098 (1986); Sardina et al., J. Org. Chem. 51, 1264 (1986); J. Org. Chem. 51, 1269 (1986); DeLuca et al., U.S. Pat. No. 5,086,191; DeLuca et al., U.S. Pat. No. 5,536,713].

Hydrindanones of the general structure II are known, or can be prepared by known methods. Specific important examples of such known bicyclic ketones are the structures with the side chains (a), (b), (c) and (d) described above, i.e. 25-hydroxy Grundmann's ketone (f) [Baggiolini et al., J. Org. Chem, 51, 3098 (1986)]; Grundmann's ketone (g) [Inhoffen et al., Chem. Ber. 90, 664 (1957)]; 25-hydroxy Windaus ketone (h) [Baggiolini et al., J. Org. Chem., 51, 3098 (1986)] and Windaus ketone (i) [Windaus et al., Ann., 524, 297 (1936)]:





For the preparation of the required phosphine oxides of general structure III, a new synthetic route has been developed starting from methyl quinate derivative 1, easily obtained from commercial (1R,3R,4S,5R)-(-)-quinic acid as described by Perlman et al.,
5 Tetrahedron Lett. 32, 7663 (1991) and DeLuca et al., U.S. Pat. No. 5,086,191. The overall process of transformation of the starting methyl ester 1 into the desired A-ring synthons, is summarized by the SCHEME I. Thus, the secondary 4-hydroxyl group of 1 was oxidized with RuO₄ (a catalytic method with RuCl₃ and NaIO₄ as co-oxidant). Use of such a strong
10 oxidant was necessary for an effective oxidation process of this very hindered hydroxyl. However, other more commonly used oxidants can also be applied (e.g. pyridinium dichromate), although the reactions usually require much longer time for completion. Second step of the synthesis comprises the Wittig reaction of the sterically hindered 4-keto compound 2 with ylide prepared from methyltriphenylphosphonium bromide and *n*-
15 butyllithium. Other bases can be also used for the generation of the reactive methylenephosphorane, like *t*-BuOK, NaNH₂, NaH, K/HMPT, NaN(TMS)₂, etc. For the preparation of the 4-methylene compound 3 some described modifications of the Wittig process can be used, e.g. reaction of 2 with activated methylenetriphenyl-phosphorane [Corey et al., Tetrahedron Lett. 26, 555 (1985)]. Alternatively, other methods widely used
20 for methylenation of unreactive ketones can be applied, e.g. Wittig-Horner reaction with the PO-ylid obtained from methyldiphenylphosphine oxide upon deprotonation with *n*-butyllithium [Schosse et al., Chimia 30, 197 (1976)], or reaction of ketone with sodium methylsulfinate [Corey et al., J. Org. Chem. 28, 1128 (1963)] and potassium methylsulfinate [Greene et al., Tetrahedron Lett. 3755 (1976)]. Reduction of the ester 3

with lithium aluminum hydride or other suitable reducing agent (e.g. DIBALH) provided the diol 4 which was subsequently oxidized by sodium periodate to the cyclohexanone derivative 5. The next step of the process comprises the Peterson reaction of the ketone 5 with methyl(trimethylsilyl)acetate. The resulting allylic ester 6 was treated with diisobutylaluminum hydride and the formed allylic alcohol 7 was in turn transformed to the desired A-ring phosphine oxide 8. Conversion of 7 to 8 involved 3 steps, namely, *in situ* tosylation with *n*-butyllithium and *p*-toluenesulfonyl chloride, followed by reaction with diphenylphosphine lithium salt and oxidation with hydrogen peroxide.

Several 2-methylene-19-nor-vitamin D compounds of the general structure IV may be synthesized using the A-ring synthon 8 and the appropriate Windaus-Grundmann ketone II having the desired side chain structure. Thus, for example, Wittig-Horner coupling of the lithium phosphinoxy carbanion generated from 8 and *n*-butyllithium with the protected 25-hydroxy Grundmann's ketone 9 prepared according to published procedure [Sicinski et al., J. Med. Chem. 37, 3730 (1994)] gave the expected protected vitamin compound 10. This, after deprotection with AG 50W-X4 cation exchange resin afforded 1 α ,25-dihydroxy-2-methylene-19-nor-vitamin D₃ (11).

The final step of the process was the selective homogeneous catalytic hydrogenation of the exomethylene unit at carbon 2 in the vitamin 11 performed efficiently in the presence of tris(triphenylphosphine)rhodium(I) chloride [Wilkinson's catalyst, (Ph₃P)₃RhCl]. Such reduction conditions allowed to reduce only C(2)=CH₂ unit leaving C(5)-C(8) butadiene moiety unaffected. The isolated material is an epimeric mixture (ca. 1:1) of 2-methyl-19-nor-vitamins 12 and 13 differing in configuration at C-2. The mixture can be used without separation or, if desired, the individual 2 α - and 2 β -isomers can be separated by an efficient HPLC system.

A similar chemoselectivity was also observed in hydroboration reactions to synthesize 2-hydroxymethyl derivatives 20 and 21 (see Scheme III). For this purpose, 9-borabicyclo(3.3.1)nonane (9-BBN) was used as a reagent and reaction conditions analogous as those used by Okamura for hydroboration of simple vitamin D compounds. See J. Org. Chem. 1978, 43, 1653-1656 and J. Org. Chem. 1977, 42, 2284-2291. Since this literature precedent concerned hydroboration of 1-desoxy compounds, namely, (5E)- and (5Z)-isomers of vitamin D₂ and D₃, the process was first tested using 1 α ,25-(OH)₂D₃ as a model compound. The formed organoborane intermediate was subsequently oxidized

with basic hydrogen peroxide. Such hydroboration-oxidation conditions allowed the exclusive hydroxylation of the C(2)=CH₂ unit in the vitamin 11, leaving the intercylic C(5)=C(6)-C(7)=C(8) diene moiety unaffected. The isolated epimeric mixture of 2-hydroxymethyl derivatives 20 and 21 (ca. 1:2, 35% yield) was purified and separated by straight- and reversed-phase HPLC.

The C-20 epimerization was accomplished by the analogous coupling of the phosphine oxide 8 with protected 20(S)-25-hydroxy Grundmann's ketone 15 (SCHEME II) and provided 19-nor-vitamin 16 which after hydrolysis of the hydroxy-protecting groups gave 20(S)-1 α ,25-dihydroxy-2-methylene-19-nor-vitamin D₃ (17). Hydrogenation of 17 using Wilkinson's catalyst provided the expected mixture of the 2-methyl-19-nor-vitamin D analogs 18 and 19. Subsequent hydroboration with 9-BBN yielded 20(S)-2-hydroxymethyl derivatives 22 and 23 (see Scheme III).

As noted above, other 2-methyl-19-nor-vitamin D analogs may be synthesized by the method disclosed herein. For example, 1 α -hydroxy-2-methylene-19-nor-vitamin D₃ can be obtained by providing the Grundmann's ketone (g); subsequent reduction of the A-ring exomethylene group in the formed compound can give the corresponding epimeric mixture of 1 α -hydroxy-2-methyl-19-nor-vitamin D₃ compounds.

A number of oxa-analogs of vitamin D₃ and their synthesis are also known. For example, 20-oxa analogs are described in N. Kubodera et al, Chem. Pharm. Bull., 34, 2286 (1986), and Abe et al, FEBS Lett. 222, 58, 1987. Several 22-oxa analogs are described in E. Murayama et al, Chem. Pharm. Bull., 34, 4410 (1986), Abe et al, FEBS Lett., 226, 58 (1987), PCT International Application No. WO 90/09991 and European Patent Application, publication number 184 112, and a 23-oxa analog is described in European Patent Application, publication number 78704, as well as U.S. Patent 4,772,433.

This invention is described by the following illustrative examples. In these examples specific products identified by Arabic numerals (e.g. 1, 2, 3, etc) refer to the specific structures so identified in the preceding description and in the SCHEME I and SCHEME II.

EXAMPLE 1

Preparation of 1 α ,25-dihydroxy-2 α - and 1 α ,25-dihydroxy-2 β -methyl-19-nor-vitamin D₃ (12 and 13).

Referring first to SCHEME I the starting methyl quinate derivative 1 was obtained from commercial (-)-quinic acid as described previously [Perlman et al., Tetrahedron Lett. 32, 7663 (1991) and DeLuca et al., U.S. Pat. No. 5,086,191]. 1: mp. 82-82.5 °C (from hexane), ¹H NMR (CDCl₃) δ 0.098, 0.110, 0.142, and 0.159 (each 3H, each s, 4 x SiCH₃), 0.896 and 0.911 (9H and 9H, each s, 2 x Si-*t*-Bu), 1.820 (1H, dd, J = 13.1, 10.3 Hz), 2.02 (1H, ddd, J = 14.3, 4.3, 2.4 Hz), 2.09 (1H, dd, J = 14.3, 2.8 Hz), 2.19 (1H, ddd, J = 13.1, 4.4, 2.4 Hz), 2.31 (1H, d, J = 2.8 Hz, OH), 3.42 (1H, m; after D₂O dd, J = 8.6, 2.6 Hz), 3.77 (3H, s), 4.12 (1H, m), 4.37 (1H, m), 4.53 (1H, br s, OH).

10 (a) Oxidation of 4-hydroxy group in methyl quinate derivative 1.

(3R,5R)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-oxocyclohexanecarboxylic Acid Methyl Ester (2). To a stirred mixture of ruthenium(III) chloride hydrate (434 mg, 2.1 mmol) and sodium periodate (10.8 g, 50.6 mmol) in water (42 mL) was added a solution of methyl quinate 1 (6.09 g, 14 mmol) in CCl₄/CH₃CN (1:1, 64 mL). Vigorous stirring was continued for 8 h. Few drops of 2-propanol were added, the mixture was poured into water and extracted with chloroform. The organic extracts were combined, washed with water, dried (MgSO₄) and evaporated to give a dark oily residue (ca. 5 g) which was purified by flash chromatography. Elution with hexane/ethyl acetate (8:2) gave pure, oily 4-ketone 2 (3.4 g, 56%): ¹H NMR (CDCl₃) δ 0.054, 0.091, 0.127, and 0.132 (each 3H, each s, 4 x SiCH₃), 0.908 and 0.913 (9H and 9H, each s, 2 x Si-*t*-Bu), 2.22 (1H, dd, J = 13.2, 11.7 Hz), 2.28 (1H, ~ dt, J = 14.9, 3.6 Hz), 2.37 (1H, dd, J = 14.9, 3.2 Hz), 2.55 (1H, ddd, J = 13.2, 6.4, 3.4 Hz), 3.79 (3H, s), 4.41 (1H, t, J ~ 3.5 Hz), 4.64 (1H, s, OH), 5.04 (1H, dd, J = 11.7, 6.4 Hz); MS m/z (relative intensity) no M⁺, 375 (M⁺ - *t*-Bu, 32), 357 (M⁺ - *t*-Bu - H₂O, 47), 243 (31), 225 (57), 73 (100).

(b) Wittig reaction of the 4-ketone 2.

(3R,5R)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4-methylenecyclohexanecarboxylic Acid Methyl Ester (3). To the methyltriphenylphosphonium bromide (2.813 g, 7.88 mmol) in anhydrous THF (32 mL) at 0 °C was added dropwise *n*-BuLi (2.5 M in hexanes, 6.0 mL, 15 mmol) under argon with

stirring. Another portion of $\text{MePh}_3\text{P}^+\text{Br}^-$ (2.813 g, 7.88 mmol) was then added and the solution was stirred at 0 °C for 10 min and at room temperature for 40 min. The orange-red mixture was again cooled to 0 °C and a solution of 4-ketone 2 (1.558 g, 3.6 mmol) in anhydrous THF (16 + 2 mL) was syphoned to reaction flask during 20 min. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. The mixture was then carefully poured into brine cont. 1% HCl and extracted with ethyl acetate and benzene. The combined organic extracts were washed with diluted NaHCO_3 and brine, dried (MgSO_4) and evaporated to give an orange oily residue (ca. 2.6 g) which was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave pure 4-methylene compound 3 as a colorless oil (368 mg, 24%): ^1H NMR (CDCl_3) δ 0.078, 0.083, 0.092, and 0.115 (each 3H, each s, 4 x SiCH_3), 0.889 and 0.920 (9H and 9H, each s, 2 x $\text{Si-}t\text{-Bu}$), 1.811 (1H, dd, $J = 12.6, 11.2$ Hz), 2.10 (2H, m), 2.31 (1H, dd, $J = 12.6, 5.1$ Hz), 3.76 (3H, s), 4.69 (1H, t, $J = 3.1$ Hz), 4.78 (1H, m), 4.96 (2H, m; after D_2O 1H, br s), 5.17 (1H, t, $J = 1.9$ Hz); MS m/z (relative intensity) no M^+ , 373 ($\text{M}^+ - t\text{-Bu}$, 57), 355 ($\text{M}^+ - t\text{-Bu} - \text{H}_2\text{O}$, 13), 341 (19), 313 (25), 241 (33), 223 (37), 209 (56), 73 (100).

(c) Reduction of ester group in the 4-methylene compound 3.

[(3'R,5'R)-3',5'-Bis[(*tert*-butyldimethylsilyl)oxy]-1-hydroxy-4'-methylenecyclohexyl]methanol (4). (i) To a stirred solution of the ester 3 (90 mg, 0.21 mmol) in anhydrous THF (8 mL) lithium aluminum hydride (60 mg, 1.6 mmol) was added at 0 °C under argon. The cooling bath was removed after 1 h and the stirring was continued at 6 °C for 12 h and at room temperature for 6 h. The excess of the reagent was decomposed with saturated aq. Na_2SO_4 , and the mixture was extracted with ethyl acetate and ether, dried (MgSO_4) and evaporated. Flash chromatography of the residue with hexane/ethyl acetate (9:1) afforded unreacted substrate (12 mg) and a pure, crystalline diol 4 (35 mg, 48% based on recovered ester 3): ^1H NMR ($\text{CDCl}_3 + \text{D}_2\text{O}$) δ 0.079, 0.091, 0.100, and 0.121 (each 3H, each s, 4 x SiCH_3), 0.895 and 0.927 (9H and 9H, each s, 2 x $\text{Si-}t\text{-Bu}$), 1.339 (1H, t, $J \sim 12$ Hz), 1.510 (1H, dd, $J = 14.3, 2.7$ Hz), 2.10 (2H, m), 3.29 and 3.40 (1H and 1H, each d, $J = 11.0$ Hz), 4.66 (1H, t, $J \sim 2.8$ Hz), 4.78 (1H, m), 4.92 (1H, t, $J = 1.7$ Hz), 5.13 (1H, t, $J = 2.0$ Hz); MS m/z (relative intensity) no M^+ , 345 ($\text{M}^+ - t\text{-Bu}$, 8), 327 ($\text{M}^+ - t\text{-Bu} - \text{H}_2\text{O}$, 22), 213 (28), 195 (11), 73 (100). (ii) Diisobutylaluminum hydride

(1.5 M in toluene, 2.0 mL, 3 mmol) was added to a solution of the ester 3 (215 mg, 0.5 mmol) in anhydrous ether (3 mL) at -78 °C under argon. The mixture was stirred at -78 °C for 3 h and at -24 °C for 1.5 h, diluted with ether (10 mL) and quenched by the slow addition of 2N potassium sodium tartrate. The solution was warmed to room temperature and stirred for 15 min, then poured into brine and extracted with ethyl acetate and ether. The organic extracts were combined, washed with diluted (ca. 1%) HCl, and brine, dried (MgSO₄) and evaporated. The crystalline residue was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave crystalline diol 4 (43 mg, 24%).

(d) Cleavage of the vicinal diol 4.

(3R,5R)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-4-methylenecyclohexanone (5). Sodium periodate saturated water (2.2 mL) was added to a solution of the diol 4 (146 mg, 0.36 mmol) in methanol (9 mL) at 0 °C. The solution was stirred at 0 °C for 1 h, poured into brine and extracted with ether and benzene. The organic extracts were combined, washed with brine, dried (MgSO₄) and evaporated. An oily residue was dissolved in hexane (1 mL) and applied on a silica Sep-Pak cartridge. Pure 4-methylenecyclohexanone derivative 5 (110 mg, 82%) was eluted with hexane/ethyl acetate (95:5) as a colorless oil: ¹H NMR (CDCl₃) δ 0.050 and 0.069 (6H and 6H, each s, 4 x SiCH₃), 0.881 (18H, s, 2 x Si-*t*-Bu), 2.45 (2H, ddd, J = 14.2, 6.9, 1.4 Hz), 2.64 (2H, ddd, J = 14.2, 4.6, 1.4 Hz), 4.69 (2H, dd, J = 6.9, 4.6 Hz), 5.16 (2H, s); MS m/z (relative intensity) no M⁺, 355 (M⁺ - Me, 3), 313 (M⁺ - *t*-Bu, 100), 73 (76).

(e) Preparation of the allylic ester 6.

[(3'R,5'R)-3',5'-Bis[(*tert*-butyldimethylsilyl)oxy]-4'-methylenecyclohexylidene]acetic Acid Methyl Ester (6). To a solution of diisopropylamine (37 μL, 0.28 mmol) in anhydrous THF (200 μL) was added *n*-BuLi (2.5 M in hexanes, 113 μL, 0.28 mmol) under argon at -78 °C with stirring, and methyl(trimethylsilyl)acetate (46 μL, 0.28 mmol) was then added. After 15 min, the keto compound 5 (49 mg, 0.132 mmol) in anhydrous THF (200 + 80 μL) was added dropwise. The solution was stirred at -78 °C for 2 h and the reaction mixture was quenched with saturated NH₄Cl, poured into brine and extracted with

ether and benzene. The combined organic extracts were washed with brine, dried (MgSO₄) and evaporated. The residue was dissolved in hexane (1 mL) and applied on a silica Sep-Pak cartridge. Elution with hexane and hexane/ethyl acetate (98:2) gave a pure allylic ester 6 (50 mg, 89%) as a colorless oil: ¹H NMR (CDCl₃) δ 0.039, 0.064, and 0.076 (6H, 3H, and 3H, each s, 4 x SiCH₃), 0.864 and 0.884 (9H and 9H, each s, 2 x Si-*t*-Bu), 2.26 (1H, dd, J = 12.8, 7.4 Hz), 2.47 (1H, dd, J = 12.8, 4.2 Hz), 2.98 (1H, dd, J = 13.3, 4.0 Hz), 3.06 (1H, dd, J = 13.3, 6.6 Hz), 3.69 (3H, s), 4.48 (2H, m), 4.99 (2H, s), 5.74 (1H, s); MS m/z (relative intensity) 426 (M⁺, 2), 411 (M⁺ - Me, 4), 369 (M⁺ - *t*-Bu, 100), 263 (69).

10 (f) Reduction of the allylic ester 6.

2-[(3'R,5'R)-3',5'-Bis[(*tert*-butyldimethylsilyl)oxy]-4'-methylenecyclohexylidene]ethanol (7). Diisobutylaluminum hydride (1.5 M in toluene, 1.6 mL, 2.4 mmol) was slowly added to a stirred solution of the allylic ester 6 (143 mg, 0.33 mmol) in toluene/methylene chloride (2:1, 5.7 mL) at -78 °C under argon. Stirring was continued at -78 °C for 1 h and at -46 °C (cyclohexanone/dry ice bath) for 25 min. The mixture was quenched by the slow addition of potassium sodium tartrate (2N, 3 mL), aq. HCl (2N, 3 mL) and H₂O (12 mL), and then diluted with methylene chloride (12 mL) and extracted with ether and benzene. The organic extracts were combined, washed with diluted (ca. 1%) HCl, and brine, dried (MgSO₄) and evaporated. The residue was purified by flash chromatography. Elution with hexane/ethyl acetate (9:1) gave crystalline allylic alcohol 7 (130 mg, 97%): ¹H NMR (CDCl₃) δ 0.038, 0.050, and 0.075 (3H, 3H, and 6H, each s, 4 x SiCH₃), 0.876 and 0.904 (9H and 9H, each s, 2 x Si-*t*-Bu), 2.12 (1H, dd, J = 12.3, 8.8 Hz), 2.23 (1H, dd, J = 13.3, 2.7 Hz), 2.45 (1H, dd, J = 12.3, 4.8 Hz), 2.51 (1H, dd, J = 13.3, 5.4 Hz), 4.04 (1H, m; after D₂O dd, J = 12.0, 7.0 Hz), 4.17 (1H, m; after D₂O dd, J = 12.0, 7.4 Hz), 4.38 (1H, m), 4.49 (1H, m), 4.95 (1H, br s), 5.05 (1H, t, J = 1.7 Hz), 5.69 (1H, ~t, J = 7.2 Hz); MS m/z (relative intensity) 398 (M⁺, 2), 383 (M⁺ - Me, 2), 365 (M⁺ - Me - H₂O, 4), 341 (M⁺ - *t*-Bu, 78), 323 (M⁺ - *t*-Bu - H₂O, 10), 73 (100); exact mass calcd for C₂₇H₄₄O₃ 416.3290, found 416.3279.

(g) Conversion of the allylic alcohol 7 into phosphine oxide 8.

[2-[(3'R,5'R)-3',5'-Bis[(*tert*-butyldimethylsilyl)oxy]-4'-methylenecyclohexylidene]ethyl]diphenylphosphine Oxide (8). To the allylic alcohol 7 (105 mg, 0.263 mmol) in anhydrous THF (2.4 mL) was added *n*-BuLi (2.5 M in hexanes, 105 µL, 0.263 mmol) under argon at 0 °C. Freshly recrystallized tosyl chloride (50.4 mg, 0.264 mmol) was dissolved in anhydrous THF (480 µL) and added to the allylic alcohol-BuLi solution. The mixture was stirred at 0 °C for 5 min and set aside at 0 °C. In another dry flask with air replaced by argon, *n*-BuLi (2.5 M in hexanes, 210 µL, 0.525 mmol) was added to Ph₂PH (93 µL, 0.534 mmol) in anhydrous THF (750 µL) at 0 °C with stirring. The red solution was syphoned under argon pressure to the solution of tosylate until the orange color persisted (ca. 1/2 of the solution was added). The resulting mixture was stirred an additional 30 min at 0 °C, and quenched by addition of H₂O (30 µL). Solvents were evaporated under reduced pressure and the residue was redissolved in methylene chloride (2.4 mL) and stirred with 10% H₂O₂ at 0 °C for 1 h. The organic layer was separated, washed with cold aq. sodium sulfite and H₂O, dried (MgSO₄) and evaporated. The residue was subjected to flash chromatography. Elution with benzene/ethyl acetate (6:4) gave semicrystalline phosphine oxide 8 (134 mg, 87%): ¹H NMR (CDCl₃) δ 0.002, 0.011, and 0.019 (3H, 3H, and 6H, each s, 4 x SiCH₃), 0.855 and 0.860 (9H and 9H, each s, 2 x Si-*t*-Bu), 2.0 - 2.1 (3H, br m), 2.34 (1H, m), 3.08 (1H, m), 3.19 (1H, m), 4.34 (2H, m), 4.90 and 4.94 (1H and 1H, each s), 5.35 (1H, ~ q, J = 7.4 Hz), 7.46 (4H, m), 7.52 (2H, m), 7.72 (4H, m); MS m/z (relative intensity) no M⁺, 581 (M⁺ - 1, 1), 567 (M⁺ - Me, 3), 525 (M⁺ - *t*-Bu, 100), 450 (10), 393 (48).

(h) Wittig-Horner coupling of protected 25-hydroxy Grundmann's ketone 9 with the phosphine oxide 8.

1α,25-Dihydroxy-2-methylene-19-nor-vitamin D₃ (11). To a solution of phosphine oxide 8 (33.1 mg, 56.8 µmol) in anhydrous THF (450 µL) at 0 °C was slowly added *n*-BuLi (2.5 M in hexanes, 23 µL, 57.5 µmol) under argon with stirring. The solution turned deep orange. The mixture was cooled to -78 °C and a precooled (-78 °C) solution of protected hydroxy ketone 9 (9.0 mg, 22.8 µmol), prepared according to published procedure

[Sicinski et al., J. Med. Chem. 37, 3730 (1994)], in anhydrous THF (200 + 100 μ L) was slowly added. The mixture was stirred under argon at -78 °C for 1 h and at 0 °C for 18 h. Ethyl acetate was added, and the organic phase was washed with brine, dried (MgSO_4) and evaporated. The residue was dissolved in hexane and applied on a silica Sep-Pak cartridge, and washed with hexane/ethyl acetate (99:1, 20 mL) to give 19-nor-vitamin derivative 10 (13.5 mg, 78%). The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone 9 (2 mg), and with ethyl acetate (10 mL) to recover diphenylphosphine oxide (20 mg). For analytical purpose a sample of protected vitamin 10 was further purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system. Pure compound 10 was eluted at R_V 26 mL as a colorless oil: UV (in hexane) λ_{max} 244, 253, 263 nm; ^1H NMR (CDCl_3) δ 0.025, 0.049, 0.066, and 0.080 (each 3H, each s, 4 x SiCH_3), 0.546 (3H, s, 18- H_3), 0.565 (6H, q, $J = 7.9$ Hz, 3 x SiCH_2), 0.864 and 0.896 (9H and 9H, each s, 2 x $\text{Si-}t\text{-Bu}$), 0.931 (3H, d, $J = 6.0$ Hz, 21- H_3), 0.947 (9H, t, $J = 7.9$ Hz, 3 x SiCH_2CH_3), 1.188 (6H, s, 26- and 27- H_3), 2.00 (2H, m), 2.18 (1H, dd, $J = 12.5, 8.5$ Hz, 4 β -H), 2.33 (1H, dd, $J = 13.1, 2.9$ Hz, 10 β -H), 2.46 (1H, dd, $J = 12.5, 4.5$ Hz, 4 α -H), 2.52 (1H, dd, $J = 13.1, 5.8$ Hz, 10 α -H), 2.82 (1H, br d, $J = 12$ Hz, 9 β -H), 4.43 (2H, m, 1 β - and 3 α -H), 4.92 and 4.97 (1H and 1H, each s, $=\text{CH}_2$), 5.84 and 6.22 (1H and 1H, each d, $J = 11.0$ Hz, 7- and 6-H); MS m/z (relative intensity) 758 (M^+ , 17), 729 ($\text{M}^+ - \text{Et}$, 6), 701 ($\text{M}^+ - t\text{-Bu}$, 4), 626 (100), 494 (23), 366 (50), 73 (92).

Protected vitamin 10 (4.3 mg) was dissolved in benzene (150 μ L) and the resin (AG 50W-X4, 60 mg; prewashed with methanol) in methanol (800 μ L) was added. The mixture was stirred at room temperature under argon for 17 h, diluted with ethyl acetate/ether (1:1, 4 mL) and decanted. The resin was washed with ether (8 mL) and the combined organic phases washed with brine and saturated NaHCO_3 , dried (MgSO_4) and evaporated. The residue was purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-nor-vitamin 11 (2.3 mg, 97%) was collected at R_V 29 mL (1 $\alpha,25$ -dihydroxyvitamin D_3 was eluted at R_V 52 mL in the same system) as a white solid: UV (in EtOH) λ_{max} 243.5, 252, 262.5 nm; ^1H NMR (CDCl_3) δ 0.552 (3H, s, 18- H_3), 0.941 (3H, d, $J = 6.4$ Hz, 21- H_3), 1.222 (6H, s, 26- and 27- H_3), 2.01 (2H, m), 2.27 - 2.36 (2H, m), 2.58 (1H, m), 2.80 - 2.88

(2H, m), 4.49 (2H, m, 1 β - and 3 α -H), 5.10 and 5.11 (1H and 1H, each s, =CH₂), 5.89 and 6.37 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS m/z (relative intensity) 416 (M⁺, 83), 398 (25), 384 (31), 380 (14), 351 (20), 313 (100); exact mass calcd for C₂₇H₄₄O₃ 416.3290, found 416.3279.

5

(i) Hydrogenation of 2-methylene-19-nor-vitamin 11.

1 α ,25-Dihydroxy-2 α - and 1 α ,25-Dihydroxy-2 β -methyl-19-nor-vitamin D₃ (12 and 13).

Tris(triphenylphosphine)rhodium(I) chloride (2.3 mg, 2.5 μ mol) was added to dry benzene
10 (2.5 mL) presaturated with hydrogen. The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 45 min). A solution of vitamin 11 (1.0 mg, 2.4 μ mol) in dry benzene (0.5 mL) was then added and the reaction was allowed to proceed under a continuous stream of hydrogen for 3 h. Benzene was removed under vacuum, and hexane/ethyl acetate (1:1, 2 mL) was added to the residue. The mixture was applied on a
15 silica Sep-Pak and both 2-methyl vitamins were eluted with the same solvent system (20 mL). Further purification was achieved by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) as a solvent system. The mixture (ca. 1:1) of 2-methyl-19-nor-vitamins (2 α - and 2 β -epimers 12 and 13; 0.80 mg, 80%) gave a single peak at R_v 33 mL.

20 12 and 13: UV (in EtOH) λ_{max} 243, 251, 261.5 nm; ¹H NMR (CDCl₃) δ 0.536 and 0.548 (3H and 3H, each s, 2 x 18-H₃), 0.937 (6H, d, J = 6.3 Hz, 2 x 21-H₃), 1.133 and 1.144 (3H and 3H, each d, J ~ 6 Hz, 2 x 2-CH₃), 1.219 [12H, s, 2 x (26- and 27-H₃)], 2.60 (1H, dd, J = 13.0, 4.6 Hz), 2.80 (3H, m), 3.08 (1H, dd, J = 12.6, 4.0 Hz), 3.51 (1H, dt, J = 4.6, 10.2 Hz), 3.61 (1H, dt, J = 4.5, 9.1 Hz), 3.90 (1H, narr m), 3.96 (1H, narr m), 5.82,
25 5.87, 6.26, and 6.37 (each 1H, each d, J = 11.2 Hz); MS m/z (relative intensity) 418 (M⁺, 100), 400 (25), 385 (15), 289 (30), 245 (25).

Separation of both epimers was achieved by reversed-phase HPLC (10 mm x 25 cm Zorbax-ODS column, 4 mL/min) using methanol/water (85:15) solvent system. 2 β -

Methyl vitamin 13 (0.35 mg, 35%) was collected at R_V 41 mL and its 2 α -epimer 12 (0.34 mg, 34%) at R_V 46 mL.

12: UV (in EtOH) λ_{max} 243, 251, 261 nm; 1H NMR ($CDCl_3$) δ 0.536 (3H, s, 18- H_3), 0.937 (3H, d, J = 6.4 Hz, 21- H_3), 1.134 (3H, d, J = 6.9 Hz, 2 α - CH_3), 1.218 (6H, s, 26- and 27- H_3), 2.13 (1H, \sim t, J \sim 12 Hz, 4 β -H), 2.22 (1H, br d, J = 13 Hz, 10 β -H), 2.60 (1H, dd, J = 12.8, 4.3 Hz, 4 α -H), 2.80 (2H, m, 9 β - and 10 α -H), 3.61 (1H, m, $w/2$ = 24 Hz, 3 α -H), 3.96 (1H, m, $w/2$ = 12 Hz, 1 β -H), 5.82 and 6.37 (1H and 1H, each d, J = 11.1 Hz, 7- and 6-H); MS m/z (relative intensity) 418 (M^+ , 62), 400 (32), 385 (17), 289 (36), 271 (17), 253 (20), 245 (43), 69 (100), 59 (74); exact mass calcd for $C_{27}H_{46}O_3$ 418.3447, found 418.3441.

13: UV (in EtOH) λ_{max} 242, 250.5, 261 nm; 1H NMR ($CDCl_3$) δ 0.548 (3H, s, 18- H_3), 0.940 (3H, d, J = 6.4 Hz, 21- H_3), 1.143 (3H, d, J = 6.8 Hz, 2 β - CH_3), 1.220 (6H, s, 26- and 27- H_3), 2.34 (1H, dd, J = 13.7, 3.3 Hz, 4 β -H), 2.43 (1H, br d, J = 13.7 Hz, 4 α -H), 2.80 (1H, dd, J = 12 and 4 Hz, 9 β -H), 3.08 (1H, dd, J = 13.0, 4.2 Hz, 10 β -H), 3.51 (1H, m, $w/2$ = 25 Hz, 1 β -H), 3.90 (1H, m, $w/2$ = 11 Hz, 3 α -H), 5.87 and 6.26 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 418 (M^+ , 63), 400 (47), 385 (16), 289 (40), 271 (32), 253 (27), 245 (47), 69 (100), 59 (64); exact mass calcd for $C_{27}H_{46}O_3$ 418.3447, found 418.3436.

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EXAMPLE 2

Preparation of 20(*S*)-1 α ,25-dihydroxy-2 α - and 20(*S*)-1 α ,25-dihydroxy-2 β -methyl-19-nor-vitamin D_3 (18 and 19).

SCHEME II illustrates the preparation of protected 20(*S*)-25-hydroxy Grundmann's ketone 15, its coupling with phosphine oxide 8 (obtained as described in Example 1) and selective hydrogenation of exomethylene group in 2-methylene compound 17.

(a) Silylation of hydroxy ketone 14.

20(S)-25-[(Triethylsilyl)oxy]-des-A,B-cholestan-8-one (15). A solution of the ketone 14 (Tetronics, Inc.; 56 mg, 0.2 mmol) and imidazole (65 mg, 0.95 mmol) in anhydrous DMF (1.2 mL) was treated with triethylsilyl chloride (95 μ L, 0.56 mmol); and the mixture was stirred at room temperature under argon for 4 h. Ethyl acetate was added and water, and the organic layer was separated. The ethyl acetate layer was washed with water and brine, dried (MgSO_4) and evaporated. The residue was passed through a silica Sep-Pak cartridge in hexane/ethyl acetate (9:1), and after evaporation, purified by HPLC (9.4 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (9:1) solvent system. Pure protected hydroxy ketone 15 (55 mg, 70%) was eluted at R_V 35 mL as a colorless oil: ^1H NMR (CDCl_3) δ 0.566 (6H, q, $J = 7.9$ Hz, 3 x SiCH_2), 0.638 (3H, s, 18- H_3), 0.859 (3H, d, $J = 6.0$ Hz, 21- H_3), 0.947 (9H, t, $J = 7.9$ Hz, 3 x SiCH_2CH_3), 1.196 (6H, s, 26- and 27- H_3), 2.45 (1H, dd, $J = 11.4, 7.5$ Hz, 14 α -H).

(b) Wittig-Horner coupling of protected 20(S)-25-hydroxy Grundmann's ketone 15 with the phosphine oxide 8.

20(S)-1 α ,25-Dihydroxy-2-methylene-19-nor-vitamin D_3 (17). To a solution of phosphine oxide 8 (15.8 mg, 27.1 μ mol) in anhydrous THF (200 μ L) at 0 $^\circ\text{C}$ was slowly added *n*-BuLi (2.5 M in hexanes, 11 μ L, 27.5 μ mol) under argon with stirring. The solution turned deep orange. The mixture was cooled to -78 $^\circ\text{C}$ and a precooled (-78 $^\circ\text{C}$) solution of protected hydroxy ketone 15 (8.0 mg, 20.3 μ mol) in anhydrous THF (100 μ L) was slowly added. The mixture was stirred under argon at -78 $^\circ\text{C}$ for 1 h and at 0 $^\circ\text{C}$ for 18 h. Ethyl acetate was added, and the organic phase was washed with brine, dried (MgSO_4) and evaporated. The residue was dissolved in hexane and applied on a silica Sep-Pak cartridge, and washed with hexane/ethyl acetate (99.5:0.5, 20 mL) to give 19-nor-vitamin derivative 16 (7 mg, 45%) as a colorless oil. The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone 15 (4 mg), and with ethyl acetate (10 mL) to recover diphenylphosphine oxide (9 mg). For analytical purpose a sample of protected vitamin 16 was further purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system.

16: UV (in hexane) λ_{\max} 244, 253.5, 263 nm; ^1H NMR (CDCl_3) δ 0.026, 0.049, 0.066, and 0.080 (each 3H, each s, 4 x SiCH_3), 0.541 (3H, s, 18- H_3), 0.564 (6H, q, $J = 7.9$ Hz, 3 x SiCH_2), 0.848 (3H, d, $J = 6.5$ Hz, 21- H_3), 0.864 and 0.896 (9H and 9H, each s, 2 x $\text{Si-}t\text{-Bu}$), 0.945 (9H, t, $J = 7.9$ Hz, 3 x SiCH_2CH_3), 1.188 (6H, s, 26- and 27- H_3), 2.15 - 2.35 (4H, br m), 2.43 - 2.53 (3H, br m), 2.82 (1H, br d, $J = 12.9$ Hz, 9 β -H), 4.42 (2H, m, 1 β - and 3 α -H), 4.92 and 4.97 (1H and 1H, each s, $=\text{CH}_2$), 5.84 and 6.22 (1H and 1H, each d, $J = 11.1$ Hz, 7- and 6-H); MS m/z (relative intensity) 758 (M^+ , 33), 729 ($\text{M}^+ - \text{Et}$, 7), 701 ($\text{M}^+ - t\text{-Bu}$, 5), 626 (100), 494 (25), 366 (52), 75 (82), 73 (69).

Protected vitamin 16 (5.0 mg) was dissolved in benzene (160 μL) and the resin (AG 50W-X4, 70 mg; prewashed with methanol) in methanol (900 μL) was added. The mixture was stirred at room temperature under argon for 19 h, diluted with ethyl acetate/ether (1:1, 4 mL) and decanted. The resin was washed with ether (8 mL) and the combined organic phases washed with brine and saturated NaHCO_3 , dried (MgSO_4) and evaporated. The residue was purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-nor-vitamin 17 (2.6 mg, 95%) was collected at R_V 28 mL [(20*R*)-analog was eluted at R_V 29 mL and 1 α ,25-dihydroxyvitamin D_3 at R_V 52 mL in the same system] as a white solid: UV (in EtOH) λ_{\max} 243.5, 252.5, 262.5 nm; ^1H NMR (CDCl_3) δ 0.551 (3H, s, 18- H_3), 0.858 (3H, d, $J = 6.6$ Hz, 21- H_3), 1.215 (6H, s, 26- and 27- H_3), 1.95 - 2.04 (2H, m), 2.27 - 2.35 (2H, m), 2.58 (1H, dd, $J = 13.3, 3.7$ Hz), 2.80 - 2.87 (2H, m), 4.49 (2H, m, 1 β - and 3 α -H), 5.09 and 5.11 (1H and 1H, each s, $=\text{CH}_2$), 5.89 and 6.36 (1H and 1H, each d, $J = 11.3$ Hz, 7- and 6-H); MS m/z (relative intensity) 416 (M^+ , 100), 398 (26), 380 (13), 366 (21), 313 (31); exact mass calcd for $\text{C}_{27}\text{H}_{44}\text{O}_3$ 416.3290, found 416.3275.

(c) Hydrogenation of 2-methylene-19-nor-vitamin 17.

20(*S*)-1 α ,25-Dihydroxy-2 α - and 20(*S*)-1 α ,25-Dihydroxy-2 β -methyl-19-nor-vitamin D_3 (18 and 19). Tris(triphenylphosphine)rhodium(I) chloride (2.3 mg, 2.5 μmol) was added to dry benzene (2.5 mL) presaturated with hydrogen. The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 45 min). A solution of

vitamin 17 (1.0 mg, 2.4 μ mol) in dry benzene (0.5 mL) was then added and the reaction was allowed to proceed under a continuous stream of hydrogen for 3 h. Benzene was removed under vacuum, and hexane/ethyl acetate (1:1, 2 mL) was added to the residue.

The mixture was applied on a silica Sep-Pak and both 2-methyl vitamins were eluted with
5 the same solvent system (20 mL). Further purification was achieved by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) as a solvent system. The mixture (ca. 1:1) of 2-methyl-19-nor-vitamins (2 α - and 2 β -epimers 18 and 19; 0.43 mg, 43%) gave a single peak at R_v 31 mL.

18 and 19: UV (in EtOH) λ_{max} 243, 251, 261 nm; ¹H NMR (CDCl₃) δ 0.534 and
10 0.546 (3H and 3H, each s, 2 x 18-H₃), 0.852 and 0.857 (3H and 3H, each d, J = 6.5 Hz, 2 x 21-H₃), 1.133 (3H, d, J = 6.7 Hz, 2-CH₃), 1.143 (3H, d, J = 6.5 Hz, 2-CH₃), 1.214 [12H, s, 2 x (26- and 27-H₃)], 2.60 (1H, dd, J = 12.7, 4.5 Hz), 2.80 (3H, m), 3.08 (1H, dd, J = 13.1, 4.3 Hz), 3.51 (1H, br m; after D₂O dt, J = 4.5, 10.0 Hz), 3.61 (1H, br m; after D₂O dt, J = 4.4, 9.2 Hz), 3.90 (1H, narr m), 3.96 (1H, narr m), 5.82, 5.87, 6.26, and 6.37 (each 1H,
15 each d, J = 11.3 Hz); MS m/z (relative intensity) 418 (M⁺, 100), 400 (45), 385 (20), 289 (38), 245 (47).

Separation of both epimers was achieved by reversed-phase HPLC (10 mm x 25 cm Zorbax-ODS column, 4 mL/min) using methanol/water (85:15) solvent system. 2 β -Methyl vitamin 19 (16%) was collected at R_v 36 mL and its 2 α -epimer 18 (20%) at R_v 45
20 mL.

18: UV (in EtOH) λ_{max} 242.5, 251, 261 nm; ¹H NMR (CDCl₃) δ 0.534 (3H, s, 18-H₃), 0.852 (3H, d, J = 6.6 Hz, 21-H₃), 1.133 (3H, d, J = 6.9 Hz, 2 α -CH₃), 1.214 (6H, s, 26- and 27-H₃), 2.13 (1H, ~t, J ~ 12 Hz, 4 β -H), 2.22 (1H, br d, J = 13 Hz, 10 β -H), 2.60 (1H, dd, J = 12.8, 4.4 Hz, 4 α -H), 2.80 (2H, m, 9 β - and 10 α -H), 3.61 (1H, m, w/2 = 25 Hz, 3 α -
25 H), 3.95 (1H, m, w/2 = 11 Hz, 1 β -H), 5.82 and 6.37 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 418 (M⁺, 58), 400 (25), 385 (20), 289 (28), 271 (23),

253 (22), 245 (38), 69 (100), 59 (47)); exact mass calcd for $C_{27}H_{46}O_3$ 418.3447, found 418.3450.

19: UV (in EtOH) λ_{max} 242.5, 250.5, 261 nm; 1H NMR ($CDCl_3$) δ 0.547 (3H, s, 18- H_3), 0.857 (3H, d, J = 6.6 Hz, 21- H_3), 1.143 (3H, d, J = 6.8 Hz, 2 β - CH_3), 1.214 (6H, s, 26- and 27- H_3), 2.34 (1H, dd, J = 13.8, 3.1 Hz, 4 β -H), 2.43 (1H, br d, J = 13.8 Hz, 4 α -H), 2.80 (1H, dd, J = 12 and 4 Hz, 9 β -H), 3.08 (1H, dd, J = 12.9, 4.4 Hz, 10 β -H), 3.50 (1H, m, $w/2$ = 26 Hz, 1 β -H), 3.89 (1H, m, $w/2$ = 11 Hz, 3 α -H), 5.86 and 6.26 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 418 (M^+ , 68), 400 (47), 385 (21), 289 (33), 271 (27), 253 (26), 245 (47), 69 (100), 59 (53); exact mass calcd for $C_{27}H_{46}O_3$ 418.3447, found 418.3448.

1 α ,25-Dihydroxy-2 α - and 1 α ,25-Dihydroxy-2 β -(hydroxymethyl)-19-norvitamin D_3 (20 and 21). 9-Borabicyclo[3.3.1]nonane (0.5 M in THF, 60 μ L, 30 μ mol) was added to a solution of vitamin 11 (1.25 mg, 3 μ mol) in anhydrous THF (50 μ L) at room temperature (evolution of hydrogen was observed). After 3 h of stirring, the mixture was quenched with methanol (20 μ L), stirred for 15 min at room temperature, cooled to 0 $^{\circ}C$, and treated successively with 6 M NaOH (10 μ L, 60 μ mol) and 30% H_2O_2 (10 μ L). The mixture was heated for 1 h at 55 $^{\circ}C$, cooled, benzene and brine were added, and the organic phase was separated, dried and evaporated. The crystalline residue was dissolved in ether (0.5 mL) and kept in freezer overnight. The ether solution was carefully removed from the precipitated crystals of cyclooctanediol and evaporated. Separation of the residue was achieved by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (85:15) solvent system. Traces of unreacted substrate 11 were eluted at R_V 16 mL, whereas isomeric 2-hydroxymethyl vitamins 20 and 21 were collected at R_V 33 mL and 40 mL, respectively. Further purification of both products by reversed-phase HPLC (10 mm x 25 cm Zorbax-ODS column, 4 mL/min) using methanol/water (9:1) solvent system afforded analytically pure vitamin 20 (0.14 mg, 11%) and its 2 β -isomer 21 (0.31 mg, 24%) collected at R_V 26 mL and 23 mL, respectively.

20: UV (in EtOH) λ_{\max} 242.5, 250.5, 261 nm; ^1H NMR (CDCl_3) δ 0.536 (3H, s, 18- H_3), 0.939 (3H, d, $J = 6.4$ Hz, 21- H_3), 1.214 (6H, s, 26- and 27- H_3), 2.13 (1H, br d, $J = 13.5$ Hz, 10 β -H), 2.21 (1H, \sim t, $J = 12$ Hz, 4 β -H), 2.64 (1H, dd, $J = 12.7, 4.5$ Hz, 4 α -H), 2.80 (2H, br d, $J = 12.7$ Hz, 9 β -H), 2.90 (1H, br d, $J = 13.5$ Hz, 10 α -H), 3.95 - 4.1 (3H, br m, 2 α - CH_2OH and 3 α -H), 4.23 (1H, m, $w/2 = 11$ Hz, 1 β -H), 5.79 and 6.41 (1H and 1H, each d, $J = 11.1$ Hz, 7- and 6-H); MS m/z (relative intensity) 434 (M^+ , 37), 416 (33), 398 (16), 383 (10), 305 (12), 287 (25), 269 (26), 245 (40), 69 (100), 59 (74); exact mass calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4$ 434.3396, found 434.3397.

21: UV (in EtOH) λ_{\max} 242, 250.5, 260.5 nm; ^1H NMR (CDCl_3) δ 0.553 (3H, s, 18- H_3), 0.942 (3H, d, $J = 6.5$ Hz, 21- H_3), 1.220 (6H, s, 26- and 27- H_3), 2.31 (1H, br d, $J = 14$ Hz, 4 β -H), 2.45 (1H, br d, $J = 14$ Hz, 4 α -H), 2.79 (1H, br d, $J = 13$ Hz, 9 β -H), 3.17 (1H, dd, $J = 12.8, 4.2$ Hz, 10 β -H), 3.95 - 4.1 (3H, br m, 1 β -H and 2 β - CH_2OH), 4.17 (1H, m, $w/2 = 10$ Hz, 3 α -H), 5.89 and 6.26 (1H and 1H, each d, $J = 11.0$ Hz, 7- and 6-H); MS m/z (relative intensity) 434 (M^+ , 50), 416 (46), 398 (20), 383 (11), 305 (14), 287 (26), 269 (30), 245 (50), 69 (100), 59 (75); exact mass calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4$ 434.3396, found 434.3402.

20(*S*)-1 α ,25-Dihydroxy-2 α - and 20(*S*)-1 α ,25-Dihydroxy-2 β -(hydroxymethyl)-19-norvitamin D₃ (22 and 23). The hydroboration of 20(*S*)-vitamin 17 and subsequent oxidation of the organoborane adduct were performed using the procedure analogous to that described above for (20*R*)-epimer 11. The reaction products were separated by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (87.5:12.5) solvent system, and the isomeric 2-hydroxymethyl vitamins 22 and 23 were collected at R_v 40 mL and 47 mL, respectively. Further purification of both products by reversed-phase HPLC (10 mm x 25 cm Zorbax-ODS column, 4 mL/min) using methanol/water (9:1) solvent system afforded analytically pure vitamin 22 (9%) and its 2 β -isomer 23 (26%) collected at R_v 25 mL and 22 mL, respectively.

22: UV (in EtOH) λ_{max} 242.5, 250.5, 261 nm; ^1H NMR (CDCl_3) δ 0.532 (3H, s, 18- H_3), 0.853 (3H, d, $J = 6.6$ Hz, 21- H_3), 1.214 (6H, s, 26- and 27- H_3), 2.13 (1H, br d, $J = 13.3$ Hz, 10 β -H), 2.21 (1H, ~t, $J = 12$ Hz, 4 β -H), 2.64 (1H, dd, $J = 12.8, 4.3$ Hz, 4 α -H), 2.80 (1H, br d, $J = 12$ Hz, 9 β -H), 2.90 (1H, br d, $J = 13.3$ Hz, 10 α -H), 3.95 - 4.1 (3H, br m, 2 α - CH_2OH and 3 α -H), 4.24 (1H, m, $w/2 = 10$ Hz, 1 β -H), 5.81 and 6.43 (1H and 1H, each d, $J = 11.1$ Hz, 7- and 6-H); MS m/z (relative intensity) 434 (M^+ , 41), 416 (34), 398 (16), 383 (10), 305 (10), 287 (28), 269 (26), 245 (51), 69 (100), 59 (82); exact mass calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4$ 434.3396, found 434.3390.

23: UV (in EtOH) λ_{max} 242, 250.5, 260.5 nm; ^1H NMR (CDCl_3) δ 0.551 (3H, s, 18- H_3), 0.861 (3H, d, $J = 6.4$ Hz, 21- H_3), 1.215 (6H, s, 26- and 27- H_3), 2.31 (1H, br d, $J = 13.7$ Hz, 4 β -H), 2.45 (1H, br d, $J = 13.7$ Hz, 4 α -H), 2.80 (1H, br d, $J = 12.5$ Hz, 9 β -H), 3.17 (1H, dd, $J = 12.7, 4.2$ Hz, 10 β -H), 3.95 - 4.1 (3H, br m, 1 β -H and 2 β - CH_2OH), 4.17 (1H, m, $w/2 = 10$ Hz, 3 α -H), 5.89 and 6.26 (1H and 1H, each d, $J = 11.1$ Hz, 7- and 6-H); MS m/z (relative intensity) 434 (M^+ , 35), 416 (29), 398 (15), 383 (8), 305 (10), 287 (18), 269 (23), 245 (48), 69 (100), 59 (93); exact mass calcd for $\text{C}_{27}\text{H}_{46}\text{O}_4$ 434.3396, found 434.3408.

BIOLOGICAL ACTIVITY OF 2-METHYL-SUBSTITUTED

19-NOR-1,25-(OH) $_2$ D $_3$ COMPOUNDS AND THEIR 20S-ISOMERS

The synthesized 2-substituted vitamins were tested for their ability to bind the porcine intestinal vitamin D receptor (See Figs. 1, 3 and 5). A comparison between the natural hormone 1 α ,25-(OH) $_2$ D $_3$ and 2-methyl substituted 19-norvitamins 12, 18 and 19 shows that they are about as active as 1 α ,25-(OH) $_2$ D $_3$, while the 2 β -methyl isomer in the 20R-series 13 is 39-fold less effective. The 2 α -hydroxymethyl vitamin D analog 22 with the "unnatural" configuration at C-20 was almost equivalent to 1 α ,25-(OH) $_2$ D $_3$ with respect to receptor binding, and the isomeric 23 proved to be less potent (6-8x) than these compounds. The corresponding 2 α -hydroxymethyl analog possessing the "natural" 20R-configuration 20 exhibited about the same binding affinity as 23, whereas the 2 β -isomer 21 was ca. 8 times less effective. The foregoing results of the competitive binding

analysis show that vitamins with the axial orientation of the 1α -hydroxy group exhibit a significantly enhanced affinity for the receptor.

It might be expected from these results that all of these compounds would have equivalent biological activity. Surprisingly, however, the 2-methyl substitutions produced highly selective analogs with their primary action on bone. When given for 7 days in a chronic mode, the most potent compounds tested were a mixture of the α and β isomers of 2-methyl 19-nor-20S-1,25-(OH) $_2$ D $_3$ (Table 1). When given at 130 pmol/day, the activity of this mixture of compounds on bone calcium mobilization (serum calcium) was much higher than that of the native hormone, possibly as high as 10 or 100 times higher. Under identical conditions, twice the dose of 1,25-(OH) $_2$ D $_3$ gave a serum calcium value of 7.2 mg/100 ml, while a mixture of 2-methyl-(α and β)-19-nor-20S-1,25-(OH) $_2$ D $_3$ gave a value of 9.6 mg/100 ml of serum calcium at the 130 pmol dose. When given at 260 pmol/day, this mixture produced the astounding value of 12.2 mg/100 ml of serum calcium at the expense of bone. To show its selectivity, these compounds produced no significant change in intestinal calcium transport at 130 pmol dose level while having a strong bone calcium mobilizing activity. At the higher dose, the 2-methyl-20S mixture did produce an intestinal transport response but gave an enormous bone mobilization response. A mixture of the α and β isomers of 2-methyl-19-nor-1,25-(OH) $_2$ D $_3$ also had strong bone calcium mobilization at both dose levels but also showed no intestinal calcium transport activity. Thus, the 2-methyl- α and β derivatives given as a mixture showed strong preferential bone calcium mobilizing activity especially when the side chain was in the 20S-configuration. These results illustrate that the 2-methyl and the 20S-2-methyl derivatives of 19-nor-1,25-(OH) $_2$ D $_3$ are selective for the mobilization of calcium from bone. Table 2 illustrates the response of both intestine and serum calcium to a single large dose of the various compounds; again, supporting the conclusions derived from Table 1.

The results in Figure 2 illustrate that a mixture of the α and β derivatives of 2-methyl-19-nor-20S-1,25-(OH) $_2$ D $_3$ is extremely potent in inducing differentiation of HL-60 cells to the monocyte. The 2-methyl- α and β compounds had activity similar to 1,25-(OH) $_2$ D $_3$. These results illustrate the potential of the 2-methyl-19-nor-20S-1,25-(OH) $_2$ D $_3$ compounds as anti-cancer agents, especially against leukemia, colon cancer, breast cancer and prostate cancer, or as agents in the treatment of psoriasis.

Competitive binding of the analogs to the porcine intestinal receptor was carried out by the method described by Dame et al (Biochemistry 25, 4523-4534, 1986).

The differentiation of HL-60 promyelocytic into monocytes was determined as described by Ostrem et al (J. Biol. Chem. 262, 14164-14171, 1987).

5

Table 1. Response of Intestinal Calcium Transport and Serum Calcium (Bone Calcium Mobilization) Activity to Chronic Doses of 2-Methyl Derivatives of 19-Nor-1,25-(OH)₂D₃ and its 20S Isomers

Group	Dose (pmol/day/7 days)	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
Vitamin D Deficient	Vehicle	5.5 ± 0.2	5.1 ± 0.16
1,25-(OH) ₂ D ₃ Treated	260	6.2 ± 0.4	7.2 ± 0.5
2-Methyl (α and β)	130	5.0 ± 0.3	6.1 ± 0.1
19-Nor-1,25-(OH) ₂ D ₃	260	5.3 ± 0.6	6.7 ± 0.4
2-Methyl (α and β)	130	5.0 ± 0.9	9.6 ± 0.1
19-Nor-20S-1,25-(OH) ₂ D ₃	260	6.9 ± 0.5	12.2 ± 0.3

10

Male weanling rats were obtained from Sprague Dawley Co. (Indianapolis, IN) and fed a 0.47% calcium, 0.3% phosphorus vitamin D-deficient diet for 1 week and then given the same diet containing 0.02% calcium, 0.3% phosphorus for 2 weeks. During the last week they were given the indicated dose of compound by intraperitoneal injection in 0.1 ml 95% propylene glycol and 5% ethanol each day for 7 days. The control animals received only the 0.1ml of 95% propylene glycol, 5% ethanol. Twenty-four hours after the last dose, the rats were sacrificed and intestinal calcium transport was determined by everted sac technique as previously described and serum calcium determined by atomic absorption spectrometry on a model 3110 Perkin Elmer instrument (Norwalk, CT). There were 5 rats per group and the values represent mean ± SEM.

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Table 2. Response of Intestinal Calcium Transport and Serum Calcium (Bone Calcium Mobilization) Activity to a Single Dose of the 2-Methyl-Derivatives of 19-Nor-1,25-(OH)₂D₃ and its 20S Isomers

Group	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
-D Control	4.2 ± 0.3	4.7 ± 0.1
1,25-(OH) ₂ D ₃	5.8 ± 0.3	5.7 ± 0.2
2-Methyl (α and β mixture)-19-Nor-1,25-(OH) ₂ D ₃	3.6 ± 0.4	5.4 ± 0.1
2-Methyl (α and β mixture)-19-Nor-20S-1,25-(OH) ₂ D ₃	6.7 ± 0.6	8.1 ± 0.3

5

Male Holtzman strain weanling rats were obtained from the Sprague Dawley Co. (Indianapolis, IN) and fed the 0.47% calcium, 0.3% phosphorus diet described by Suda et al. (J. Nutr. 100, 1049-1052, 1970) for 1 week and then fed the same diet containing 0.02% calcium and 0.3% phosphorus for 2 additional weeks. At this point, they received a single intrajugular injection of the indicated dose dissolved in 0.1 ml of 95% propylene glycol/5% ethanol. Twenty-four hours later they were sacrificed and intestinal calcium transport and serum calcium were determined as described in Table 1. The dose of the compounds was 650 pmol and there were 5 animals per group. The data are expressed as mean ± SEM.

When given for 7 days in a chronic mode, the most potent individual compound tested was 2α-methyl 19-nor-20S-1,25-(OH)₂D₃ (Table 3). When given at 130 pmol/day, the activity of this compound on bone calcium mobilization (serum calcium) was much higher than that of the native hormone, possibly as high as 10 or 100 times higher. Under identical conditions, twice the dose of 1,25-(OH)₂D₃ gave a serum calcium value of 6.6 ± 0.4 mg/100 ml, while 2α-methyl-19-nor-20S-1,25-(OH)₂D₃ gave a value of 8.3 ± 0.7 mg/100 ml of serum calcium at the 130 pmol dose. When given at 260 pmol/day, 2α-methyl-19-nor-20S-1,25-(OH)₂D₃ produced the astounding value of 10.3 ± 0.11 mg/100 ml of serum calcium at the expense of bone. To show its selectivity, this compound also produced a significant change in intestinal calcium transport at both the 260 pmol and the

130 pmol dose levels while having a strong bone calcium mobilizing activity. At the higher dose, the 2 α -methyl-20S compound did produce a significant intestinal transport response but also gave an enormous bone mobilization response. With respect to the 2 β -methyl-19-nor-20S compound, the data in Table 3 show it has little, if any, intestinal calcium transport activity, and little, if any, bone mobilization activity. The data in Table 4 illustrate that 2 α -methyl-19-nor-1,25-(OH)₂D₃ also had relatively strong bone calcium mobilization at both dose levels and also showed some intestinal calcium transport activity. In contrast, 2 β -methyl-19-nor-1,25-(OH)₂D₃ showed little, if any, intestinal calcium transport or bone calcium mobilization activities. Thus, the 2 α -methyl-19-nor derivative showed strong preferential bone calcium mobilizing activity especially when the side chain was in the 20S-configuration. These results illustrate that the 2 α -methyl and the 20S-2 α -methyl derivatives of 19-nor-1,25-(OH)₂D₃ are selective for the mobilization of calcium from bone.

The results in Figure 4 illustrate that 2 α -methyl-19-nor-20S-1,25-(OH)₂D₃ and 2 α -methyl-19-nor-1,25-(OH)₂D₃ are extremely potent in inducing differentiation of HL-60 cells to the monocyte. The 2 β -methyl compounds had activity similar to 1,25-(OH)₂D₃. These results illustrate the potential of the 2 α -methyl-19-nor-20S-1,25-(OH)₂D₃ compound as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer and prostate cancer, or as an agent in the treatment of psoriasis.

Competitive binding of the analogs to the porcine intestinal receptor was carried out by the method described by Dame et al (Biochemistry 25, 4523-4534, 1986).

The differentiation of HL-60 promyelocytic into monocytes was determined as described by Ostrem et al (J. Biol. Chem. 262, 14164-14171, 1987).

Table 3. Response of Intestinal Calcium Transport and Serum Calcium (Bone Calcium Mobilization) Activity to Chronic Doses of the 20S Isomers of 2-Methyl Derivatives of 19-Nor-1,25-(OH)₂D₃

Group	Dose (pmol/day/7 days)	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
Vitamin D Deficient	Vehicle	2.9 \pm 0.2	4.2 \pm 0.1
1,25-(OH) ₂ D ₃ Treated	260	4.6 \pm 0.2	6.6 \pm 0.4

Group	Dose (pmol/day/7 days)	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
2 α -Methyl-19-nor-20(S)- 1,25-(OH) ₂ D ₃	130	12.9 \pm 1.9	8.3 \pm 0.7
	260	8.4 \pm 1.1	10.3 \pm 0.11
2 β -Methyl-19-nor-20(S)- 1,25-(OH) ₂ D ₃	130	2.9 \pm 0.3	4.4 \pm 0.1
	260	3.8 \pm 0.1	4.4 \pm 0.1

With respect to the data in Tables 3 and 4, male weanling rats were obtained from Sprague Dawley Co. (Indianapolis, IN) and fed a 0.47% calcium, 0.3% phosphorus vitamin D-deficient diet for 1 week and then given the same diet containing 0.02% calcium, 0.3% phosphorus for 2 weeks. During the last week they were given the indicated dose of compound by intraperitoneal injection in 0.1 ml 95% propylene glycol and 5% ethanol each day for 7 days. The control animals received only the 0.1ml of 95% propylene glycol, 5% ethanol. Twenty-four hours after the last dose, the rats were sacrificed and intestinal calcium transport was determined by everted sac technique as previously described and serum calcium determined by atomic absorption spectrometry on a model 3110 Perkin Elmer instrument (Norwalk, CT). There were 5 rats per group and the values represent mean \pm SEM.

Table 4. Response of Intestinal Calcium Transport and Serum Calcium (Bone Calcium Mobilization) Activity to Chronic Doses of the 2-Methyl-Derivatives of 19-Nor-1,25-(OH)₂D₃

Group	Dose Pmol/day/7 days	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
-D Control	0	2.3 \pm 0.8	3.9 \pm 0.2
1,25-(OH) ₂ D ₃	260	5.6 \pm 1.3	6.1 \pm 0.5
2 α -Methyl-19-nor- 1,25-(OH) ₂ D ₃	130	4.3 \pm 1.0	4.8 \pm 0.2
	260	5.3 \pm 1.3	5.8 \pm 0.5
2 β -Methyl-19-nor- 1,25-(OH) ₂ D ₃	130	4.4 \pm 0.8	4.1 \pm 0.1
	260	3.1 \pm 0.9	3.8 \pm 0.2

Table 5 provides intestinal calcium transport and bone calcium mobilization data for 2-hydroxymethyl derivatives of 19-nor-1 α ,25-(OH) $_2$ D $_3$. These derivatives turned out to be relatively inactive, including those in the 20S-series 22 and 23.

5 Table 5. Response of Intestinal Calcium Transport and Serum Calcium (Bone Calcium Mobilization) Activity to Chronic Doses of the 2-Hydroxymethyl-Derivatives of 19-Nor-1,25-(OH) $_2$ D $_3$

Group	Dose Pmol/day/7 days	Intestinal Calcium Transport (S/M)	Serum Calcium (mg/100 ml)
Vitamin D Deficient	Vehicle	4.0 \pm 0.3	3.8 \pm 0.1
1,25-(OH) $_2$ D $_3$ Treated	260	6.6 \pm 0.5	5.2 \pm 0.1
2 α -Hydroxymethyl-19-	130	5.0 \pm 0.3	4.0 \pm 0.1
nor-20(S)-1,25-(OH) $_2$ D $_3$	260	5.8 \pm 0.4	3.9 \pm 0.1
2 β -Hydroxymethyl-19-	130	3.5 \pm 0.7	3.6 \pm 0.1
nor-20(S)-1,25-(OH) $_2$ D $_3$	260	3.5 \pm 0.3	3.5 \pm 0.2

10 In the next assay, the cellular activity of the synthesized compounds was established by studying their ability to induce differentiation of human promyelocyte HL-60 cells into monocytes. It was found that all of the synthesized vitamin D analogs with the "unnatural" 20S-configuration were more potent than 1 α ,25-(OH) $_2$ D $_3$. Moreover, the same relationship between cellular activity and conformation of the vitamin D compounds

15 was established as in the case of receptor binding analysis and in vivo studies, i.e. 2 α -substituted vitamin D analogs were considerably more active than their 2 β -substituted counterparts with the equatorially oriented 1 α -hydroxy group. Thus, 2 α -methyl vitamins 12 and 18 proved to be 100 and 10 times, respectively, more active than their corresponding 2 β -isomers 13 and 19 in the cultures of HL-60 in vitro, whereas in the case

20 of 2-hydroxymethyl derivatives (20, 22 versus 21, 23) these differences were slightly smaller. Since vitamins with 2 β -methyl substituent (13, 19) and both 2-hydroxymethyl analogs in 20S-series (22, 23) have selective activity profiles combining high potency in cellular differentiation, and lack of calcemic activity, such compounds are potentially useful as therapeutic agents for the treatment of cancer.

These results indicate that variation of substituents on C-2 in the parent 19-nor-1 α ,25-dihydroxyvitamin D₃ can change completely (and selectively) the biological potency of the analogs. These results suggest that 2 α -methyl-19-nor-20S-1,25-(OH)₂D₃ has preferential activity on bone, making it a candidate for treatment of bone disease.

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EXAMPLE 3

Preparation of 20(S)-1 α ,25-Dihydroxy-2 α -and 20(S)-1 α ,25-Dihydroxy-2 β -methyl-26,27-dihomo-19-norvitamin D₃ (36 and 37). Reference is made to SCHEME IV.

10 20(S)-25-[(Triethylsilyl)oxy]-des-A,B-26,27-dihomocholestan-8-one (32). To a solution of 20(S)-25-hydroxy Grundmann's ketone analog 31 (*Tetrionics*, Madison, WI; 18.5 mg, 0.06 mmol) in anhydrous CH₂Cl₂ (60 μ L) was added 2,6-lutidine (17.4 μ L, 0.15 mmol) and triethylsilyl trifluoromethanesulfonate (20.3 μ L, 0.09 mmol). The mixture was stirred at room temperature under argon for 1 h. Benzene was added and water, and the
15 organic layer was separated, washed with sat. CuSO₄ and water, dried (MgSO₄) and evaporated. The oily residue was redissolved in hexane and applied on a silica Sep-Pak cartridge (2 g). Elution with hexane (10 mL) gave a small quantity of less polar compounds; further elution with hexane/ethyl acetate (9:1) provided the silylated ketone. Final purification was achieved by HPLC (10-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (95:5) solvent system. Pure protected hydroxy ketone 32 (16.7
20 mg, 66%) was eluted at R_V 37 mL as a colorless oil: ¹H NMR (CDCl₃) δ 0.573 (6H, q, J = 7.9 Hz, 3 x SiCH₂), 0.639 (3H, s, 18-H₃), 0.825 (6H, t, J = 7.5 Hz, 26- and 27-CH₃), 0.861 (3H, d, J = 6.1 Hz, 21-H₃), 0.949 (9H, t, J = 7.9 Hz, 3 x SiCH₂CH₃), 2.45 (1H, dd, J = 11.4, 7.6 Hz, 14 α -H).

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20(S)-1 α ,25-Dihydroxy-2-methylene-26,27-dihomo-19-norvitamin D₃ (35). To a solution of phosphine oxide 33 (9.1 mg, 15.6 μ mol) in anhydrous THF (150 μ L) at 0 °C was slowly added *n*-BuLi (2.5 M in hexanes, 7 μ L, 17.5 μ mol) under argon with stirring. The solution turned deep orange. It was stirred for 10 min at 0 °C, then cooled to -78 °C
30 and a precooled (-78 °C) solution of protected hydroxy ketone 32 (16.5 mg, 39.0 μ mol) in anhydrous THF (300 + 100 μ L) was slowly added. The mixture was stirred under argon at

-78 °C for 1.5 h and at 0 °C for 19 h. Water and ethyl acetate were added, and the organic phase was washed with brine, dried (MgSO₄) and evaporated. The residue was dissolved in hexane and applied on a silica Sep-Pak cartridge, and washed with hexane/ethyl acetate (99.7:0.3, 20 mL) to give slightly impure 19-norvitamin derivative 34 (ca. 4 mg). The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone (contaminated with 14 β -isomer), and with ethyl acetate (10 mL) to recover diphenylphosphine oxide 33 (ca. 6 mg) that was subsequently purified by HPLC (10-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system; pure compound 33 (5.1 mg) was eluted at R_V 36 mL. The protected vitamin 34 was further purified by HPLC (6.2-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system. Pure compound 34 (3.6 mg, 67% yield considering the recovery of unreacted 33) was eluted at R_V 19 mL as a colorless oil: UV (in hexane) λ_{max} 244.0, 252.5, 262.5 nm; ¹H NMR (CDCl₃) δ 0.026, 0.048, 0.066, and 0.079 (each 3H, each s, 4 x SiCH₃), 0.544 (3H, s, 18-H₃), 0.570 (6H, q, J = 7.9 Hz, 3 x SiCH₂), 0.821 (6H, t, J = 7.5 Hz, 26- and 27-CH₃), 0.849 (3H, d, J = 6.7 Hz, 21-H₃), 0.864 and 0.896 (9H and 9H, each s, 2 x Si-*t*-Bu), 0.946 (9H, t, J = 7.9 Hz, 3 x SiCH₂CH₃), 1.99 (2H, m), 2.18 (1H, dd, J = 12.6, 8.2 Hz, 4 β -H), 2.34 (1H, dd, J = 13.0, 2.9 Hz, 10 β -H), 2.46 (1H, dd, J = 12.6, 4.3 Hz, 4 α -H), 2.51 (1H, dd, J = 13.0, 6.2 Hz, 10 α -H), 2.82 (1H, br d, J = 12 Hz, 9 β -H), 4.43 (2H, m, 1 β - and 3 α -H), 4.92 and 4.97 (1H and 1H, each s, =CH₂), 5.84 and 6.22 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 786 (M⁺, 15), 757 (M⁺ - Et, 22), 729 (M⁺ - *t*-Bu, 5), 654 (100), 522 (15), 366 (43), 201 (31).

Protected vitamin 34 (3.5 mg) was dissolved in benzene (150 μ L) and the resin (AG 50W-X4, 40 mg; prewashed with methanol) in methanol (550 μ L) was added. The mixture was stirred at room temperature under argon for 14 h, diluted with ethyl acetate/ether (1:1, 4 mL) and decanted. The resin was washed with ether (8 mL) and the combined organic phases washed with brine and saturated NaHCO₃, dried (MgSO₄) and evaporated. The residue was purified by HPLC (6.2-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. Analytically pure 2-methylene-19-norvitamin 35 (1.22 mg, 62%) was collected at R_V 21 mL as a white solid: UV (in EtOH) λ_{max} 243.5, 252.0, 262.0 nm; ¹H NMR (CDCl₃) δ 0.550 (3H, s, 18-H₃), 0.855 (3H,

d, $J = 6.8$ Hz, 21- H_3), 0.860 (6H, t, $J = 7.5$ Hz, 26- and 27- CH_3), 2.00 (3H, m), 2.30 (1H, dd, $J = 13.3, 8.6$ Hz, 10 α -H), 2.33 (1H, dd, $J = 13.3, 6.3$ Hz, 4 β -H), 2.58 (1H, dd, $J = 13.3, 3.9$ Hz, 4 α -H), 2.82 (1H, br d, $J = 12$ Hz, 9 β -H), 2.85 (1H, dd, $J = 13.3, 4.7$ Hz, 10 β -H), 4.48 (2H, m, 1 β - and 3 α -H), 5.09 and 5.11 (1H and 1H, each s, = CH_2), 5.89 and 6.36 (1H and 1H, each d, $J = 11.3$ Hz, 7- and 6-H); MS m/z (relative intensity) 444 (M^+ , 100), 426 (35), 408 (11), 397 (19), 379 (32), 341 (31), 287 (32), 273 (43), 269 (28), 251 (22); exact mass calcd for $C_{29}H_{48}O_3$ 444.3603, found 444.3602.

20(*S*)-1 α ,25-Dihydroxy-2 α - and 20(*S*)-1 α ,25-Dihydroxy-2 β -methyl-26,27-dihomo-19-norvitamin D₃ (36 and 37). Tris(triphenylphosphine)rhodium (I) chloride (2.3 mg, 2.5 μ mol) was added to dry benzene (2.5 mL) presaturated with hydrogen. The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 45 min). A solution of vitamin 35 (1.0 mg, 2.3 μ mol) in dry benzene (0.5 mL) was then added and the reaction was allowed to proceed under a continuous stream of hydrogen for 4.5 h.

15 A new portion of the catalyst (2.3 mg, 2.5 μ mol) was added and hydrogen was passed for additional 1 h. Benzene was removed under vacuum, the residue was redissolved in hexane/ethyl acetate (1:1, 2 mL) and applied on Waters silica Sep-Pak. A mixture of 2-methyl vitamins was eluted with the same solvent system (20 mL). The compounds were further purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using

20 hexane/2-propanol (9:1) solvent system. The mixture (ca. 1:1) of 2-methyl-19-norvitamins 36 and 37 (0.37 mg, 37%) gave a single peak at R_V 23 mL. Separation of both epimers was achieved by reversed-phase HPLC (6.2-mm x 25-cm Zorbax-ODS column, 2 mL/min) using methanol/water (85:15) solvent system. 2 β -Methyl vitamin 37 was collected at R_V 21 mL and its 2 α -epimer 36 at R_V 27 mL.

25 36: UV (in EtOH) λ_{max} 242.5, 251.0, 261.0 nm; 1H NMR ($CDCl_3$) δ 0.534 (3H, s, 18- H_3), 0.851 (3H, d, $J \sim 7$ Hz, 21- H_3), 0.858 (6H, t, $J = 7.5$ Hz, 26- and 27- CH_3), 1.133 (3H, d, $J = 6.9$ Hz, 2 α - CH_3), 2.13 (1H, \sim t, $J \sim 12$ Hz, 4 β -H), 2.23 (1H, br d, $J = 13.4$ Hz, 10 β -H), 2.60 (1H, dd, $J = 13.1, 4.4$ Hz, 4 α -H), 2.80 (2H, m, 9 β - and 10 α -H), 3.61 (1H, m, $w/2 = 26$ Hz, 3 α -H), 3.96 (1H, m, $w/2 = 13$ Hz, 1 β -H), 5.82 and 6.37 (1H and 1H, each d, $J =$

30 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 446 (M^+ , 53), 428 (46), 410 (12), 399

(35), 381 (17), 289 (35), 273 (48), 271 (30), 253 (24), 69 (100); exact mass calcd for $C_{29}H_{50}O_3$ 446.3760, found 446.3758.

37: UV (in EtOH) λ_{\max} 242.5, 251.0, 261.0 nm; 1H NMR ($CDCl_3$) δ 0.546 (3H, s, 18- H_3), 0.855 (3H, d, J = 6.8 Hz, 21- H_3), 0.860 (6H, t, J = 7.4 Hz, 26- and 27- CH_3), 1.143 (3H, d, J = 6.9 Hz, 2 β - CH_3), 2.34 (1H, dd, J = 13.7, 3.3 Hz, 4 β -H), 2.43 (1H, br d, J = 13.7 Hz, 4 α -H), 2.80 (1H, dd, J = 12 and 4 Hz, 9 β -H), 3.08 (1H, dd, J = 12.8, 4.1 Hz, 10 β -H), 3.50 (1H, m, $w/2$ = 26 Hz, 1 β -H), 3.90 (1H, m, $w/2$ = 11 Hz, 3 α -H), 5.87 and 6.26 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 446 (M^+ , 39) 428 (46), 410 (12), 399 (30), 381 (17), 289 (37), 273 (50), 271 (31), 253 (27), 69 (100); exact mass calcd for $C_{29}H_{50}O_3$ 446.3760, found 446.3740.

Biological Activity of 20(S)-1 α ,25-Dihydroxy-2 α -methyl-26,27-dihomo-19-nor-vitamin D_3 and 20(S)-1 α ,25-Dihydroxy-2 β -methyl-26,27-dihomo-19-norvitamin D_3 (36 and 37).

Competitive binding of the analogs to the porcine intestinal receptor was carried out by the method described by Dame et al (Biochemistry 25, 4523-4534, 1986).

The differentiation of HL-60 promyelocytic into monocytes was determined as described by Ostrem et al (J. Biol. Chem. 262 14164-14171, 1987).

Table 6. VDR Binding Properties^a and HL-60 Differentiating Activities^b of 2-Substituted Analogs of 20(S)-1 α ,25-Dihydroxy-26,27-dihomo-19-norvitamin D_3

Compound	Compd. no.	VDR Binding		HL-60 Differentiation	
		ED ₅₀ (M)	Binding ratio	ED ₅₀ (M)	Activity ratio
1 α ,25-(OH) ₂ D_3		8.7×10^{-10}	1	4.0×10^{-9}	1
2 α -methyl-26,27-dihomo-19-nor-20(S)-1 α ,25-(OH) ₂ D_3	36	3.1×10^{-9}	3.6	6.0×10^{-11}	0.01
2 β -methyl-26,27-dihomo-19-nor-20(S)-1 α ,25-(OH) ₂ D_3	37	4.8×10^{-9}	5.5	1.1×10^{-10}	0.03

^aCompetitive binding of $1\alpha,25-(\text{OH})_2\text{D}_3$ and the synthesized vitamin D analogs to the porcine intestinal vitamin D receptor. The experiments were carried out in triplicate on two different occasions. The ED₅₀ values are derived from dose-response curves and represent the analog concentration required for 50% displacement of the radiolabeled $1\alpha,25-(\text{OH})_2\text{D}_3$ from the receptor protein. Binding ratio is the ratio of the analog average ED₅₀ to the ED₅₀ for $1\alpha,25-(\text{OH})_2\text{D}_3$.

^bInduction of differentiation of HL-60 promyelocytes to monocytes by $1\alpha,25-(\text{OH})_2\text{D}_3$ and the synthesized vitamin D analogs. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated three times. The values ED₅₀ are derived from dose-response curves and represent the analog concentration capable of inducing 50% maturation. Differentiation activity ratio is the ratio of the analog average ED₅₀ to the ED₅₀ for $1\alpha,25-(\text{OH})_2\text{D}_3$.

Table 7. Support of Intestinal Calcium Transport and Bone Calcium Mobilization by 2-Substituted Analogs of $20(\text{S})-1\alpha,25\text{-Dihydroxy-}26,27\text{-dihomo-}19\text{-norvitamin D}_3$ in Vitamin D-Deficient Rats on a Low-Calcium Diet^a

Compound	Compd. no.	Amount (pmol)	Ca Transport S/M (mean \pm SEM)	Serum Ca (mean \pm SEM)
one (control)		0	2.7 ± 0.3^b	4.7 ± 0.2^b
$1\alpha,25-(\text{OH})_2\text{D}_3$		260	7.2 ± 0.6^c	5.6 ± 0.2^c
25 $2\alpha\text{-methyl-}26,27\text{-dihomo-}19\text{-nor-}20(\text{S})\text{-}1\alpha,25-(\text{OH})_2\text{D}_3$	36	32	5.8 ± 0.4^{d1}	5.9 ± 0.2^{d1}
		65	8.4 ± 0.8^{d2}	9.3 ± 0.2^{d2}
none (control)		0	3.6 ± 0.4^b	5.0 ± 0.1^b
$1\alpha,25-(\text{OH})_2\text{D}_3$		260	5.0 ± 0.4^c	6.3 ± 0.2^c
30 $2\beta\text{-methyl-}26,27\text{-dihomo-}19\text{-nor-}20(\text{S})\text{-}1\alpha,25-(\text{OH})_2\text{D}_3$	37	65	4.7 ± 0.6^{d1}	5.0 ± 0.0^{d1}
		260	5.2 ± 0.6^{d2}	9.9 ± 0.3^{d2}

^aWeanling male rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 consecutive days. All doses were administered intraperitoneally in 0.1 ml propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were at least 6 rats per group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (S/M), panel 1, *b* from *c*, and *d*², *p* < 0.001, *b* from *d*¹, *p* = 0.001; panel 2, *b* from *c*, *d*¹, and *d*², *p* < 0.05; serum calcium, panel 1, *b* from *c* and *d*¹, *p* < 0.05, *b* from *d*², *p* < 0.001; panel 2, *b* from *c*, *p* < 0.01, *b* from *d*¹, NS, *b* from *d*², *p* < 0.001.

EXAMPLE 4

Preparation of 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-2 α -methyl-19-norvitamin D₃ and 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-2 β -methyl-19-norvitamin D₃ (48 and 49). Reference is made to SCHEMES V and VI.

20(S)-25-[(Triethylsilyl)oxy]-des-A,B-26,27-dimethylene-cholestan-8-one (42). To a solution of 20(S)-25-hydroxy Grundmann's ketone analog 41 (*Tetrionics*, Madison, WI; 15.0 mg, 0.049 mmol) in anhydrous CH₂Cl₂ (50 μ L) was added 2,6-lutidine (15 μ L, 0.129 mmol) and triethylsilyl trifluoromethanesulfonate (17.0 μ L, 0.075 mmol). The mixture was stirred at room temperature under argon for 1 h. Benzene was added and water, and the organic layer was separated, washed with sat. CuSO₄ and water, dried (MgSO₄) and evaporated. The oily residue was redissolved in hexane and applied on a silica Sep-Pak cartridge (2 g). Elution with hexane (10 mL) gave a small quantity of less polar compounds; further elution with hexane/ethyl acetate (9:1) provided the silylated ketone. Final purification was achieved by HPLC (10-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (95:5) solvent system. Pure protected hydroxy ketone 42 (9.4 mg, 46%) was eluted at R_V 39 mL as a colorless oil: ¹H NMR (CDCl₃) δ 0.576 (6H, q, J = 7.9 Hz, 3 x SiCH₂), 0.638 (3H, s, 18-H₃), 0.865 (3H, d, J = 6.1 Hz, 21-H₃), 0.949 (9H, t, J = 7.9 Hz, 3 x SiCH₂CH₃), 2.45 (1H, dd, J = 11.4, 7.5 Hz, 14 α -H).

20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-2-methylene-19-norvitamin D₃ (47).

To a solution of phosphine oxide 43 (17.7 mg, 30.4 μ mol) in anhydrous THF (300 μ L) at 0 °C was slowly added *n*-BuLi (2.5 M in hexanes, 13 μ L, 32.5 μ mol) under argon with stirring. The solution turned deep orange. It was stirred for 10 min at 0 °C, then cooled to -78 °C and a precooled (-78 °C) solution of protected hydroxy ketone 41 (17.8 mg, 42.3 μ mol) in anhydrous THF (300 + 100 μ L) was slowly added. The mixture was stirred under argon at -78 °C for 1.5 h and at 0 °C for 18 h. Water and ethyl acetate were added, and the organic phase was washed with brine, dried (MgSO₄) and evaporated. The residue was dissolved in hexane and applied on a silica Sep-Pak cartridge, and washed with hexane/ethyl acetate (99.7:0.3, 20 mL) to give slightly impure 19-norvitamin derivative 44 (ca. 11 mg). The Sep-Pak was then washed with hexane/ethyl acetate (96:4, 10 mL) to recover some unchanged C,D-ring ketone (contaminated with 14 β -isomer), and with ethyl acetate (10 mL) to recover diphenylphosphine oxide 43 (ca. 8 mg) that was subsequently purified by HPLC (10-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system; pure compound 43 (7.6 mg) was eluted at R_V 36 mL. The protected vitamin 44 was further purified by HPLC (6.2-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/ethyl acetate (99.9:0.1) solvent system. Pure compound 44 (10.1 mg, 74% yield considering the recovery of unreacted 43) was eluted at R_V 27 mL as a colorless oil: UV (in hexane) λ_{max} 244.0, 252.5, 262.5 nm; ¹H NMR (CDCl₃) δ 0.027, 0.048, 0.067, and 0.080 (each 3H, each s, 4 x SiCH₃), 0.544 (3H, s, 18-H₃), 0.575 (6H, q, J = 7.9 Hz, 3 x SiCH₂), 0.854 (3H, d, J = 6.1 Hz, 21-H₃), 0.866 and 0.896 (9H and 9H, each s, 2 x Si-*t*-Bu), 0.947 (9H, t, J = 7.9 Hz, 3 x SiCH₂CH₃), 1.99 (2H, m), 2.18 (1H, dd, J = 12.8, 8.6 Hz, 4 β -H), 2.34 (1H, dd, J = 13.2, 2.7 Hz, 10 β -H), 2.46 (1H, dd, J = 12.8, 4.4 Hz, 4 α -H), 2.51 (1H, dd, J = 13.2, 6.0 Hz, 10 α -H), 2.82 (1H, br d, J = 12 Hz, 9 β -H), 4.42 (2H,

m, 1 β - and 3 α -H), 4.92 and 4.97 (1H and 1H, each s, =CH₂), 5.84 and 6.22 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 784 (M⁺, 8), 755 (M⁺ - Et, 4), 727 (M⁺ - *t*-Bu, 6), 652 (100), 520 (31), 366 (49), 199 (23).

- Protected vitamin 44 (7.0 mg) was dissolved in benzene (220 μ L) and the resin (AG 50W-X4, 95 mg; prewashed with methanol) in methanol (1.2 mL) was added. The mixture was stirred at room temperature under argon for 21 h, diluted with ethyl acetate/ether (1:1, 4 mL) and decanted. The resin was washed with ether (10 mL) and the combined organic phases washed with brine and saturated NaHCO₃, dried (MgSO₄) and evaporated. The residue was separated by HPLC (6.2-mm x 25-cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system and the following analytically pure 2-methylene-19-norvitamins were isolated: 1 α -hydroxy-25-dehydrovitamin 45 (0.68 mg, 17%) was collected at R_v 13 mL, 1 α -hydroxy-25-methoxyvitamin 46 (0.76 mg, 19%) was collected at R_v 16 mL and 1 α ,25-dihydroxyvitamin 47 (2.0 mg, 51%) was collected at R_v 21 mL.
- 45: UV (in EtOH) λ_{max} 243.5, 251.5, 262.0 nm; ¹H NMR (CDCl₃) δ 0.542 (3H, s, 18-H₃), 0.847 (3H, d, J = 6.5 Hz, 21-H₃), 1.93-2.07 (4H, m), 2.18-2.25 (2H, m), 2.26-2.36 (4H, m), 2.58 (1H, dd, J = 13.3, 3.9 Hz, 4 α -H), 2.82 (1H, br d, J = 13 Hz, 9 β -H), 2.85 (1H, dd, J = 13.3, 4.5 Hz, 10 β -H), 4.48 (2H, m, 1 β - and 3 α -H), 5.09 and 5.11 (1H and 1H, each s, =CH₂), 5.32 (1H, m, w/2 = 7 Hz, 24-H), 5.88 and 6.36 (1H and 1H, each d, J = 11.1 Hz, 7- and 6-H); MS m/z (relative intensity) 424 (M⁺, 100), 406 (7), 339 (16), 287 (16), 271 (24), 269 (17), 251 (12); exact mass calcd for C₂₉H₄₄O₂ 424.3341, found 424.3343.
- 46: UV (in EtOH) λ_{max} 243.5, 252.0, 262.0 nm; ¹H NMR (CDCl₃) δ 0.553 (3H, s, 18-H₃), 0.858 (3H, d, J = 6.5 Hz, 21-H₃), 1.95-2.05 (2H, m), 2.30 (1H, dd, J = 13.3, 8.3 Hz, 10 α -H), 2.33 (1H, dd, J = 13.4, 6.0 Hz, 4 β -H), 2.58 (1H, dd, J = 13.4, 3.8 Hz, 4 α -H), 2.82 (1H, br d, J = 13 Hz, 9 β -H), 2.85 (1H, dd, J = 13.3, 4.4 Hz, 10 β -H), 3.13 (3H, s, OCH₃), 4.48 (2H, m, 1 β - and 3 α -H), 5.09 and 5.11 (1H and 1H, each s, =CH₂), 5.89 and 6.36 (1H and 1H, each d, J = 11.2 Hz, 7- and 6-H); MS m/z (relative intensity) 456 (M⁺, 54), 424 (27),

406 (12), 339 (16), 287 (13), 271 (41), 99 (100); exact mass calcd for $C_{30}H_{48}O_3$ 456.3603, found 456.3603.

47: UV (in EtOH) λ_{max} 243.5, 252.0, 262.0 nm; 1H NMR ($CDCl_3$) δ 0.551 (3H, s, 18- H_3), 0.859 (3H, d, J = 6.6 Hz, 21- H_3), 1.95-2.05 (2H, m), 2.30 (1H, dd, J = 13.5, 8.4 Hz, 10 α -H), 2.33 (1H, dd, J = 13.3, 6.3 Hz, 4 β -H), 2.58 (1H, dd, J = 13.3, 4.0 Hz, 4 α -H), 2.82 (1H, br d, J = 12 Hz, 9 β -H), 2.85 (1H, dd, J = 13.5, 4.4 Hz, 10 β -H), 4.48 (2H, m, 1 β - and 3 α -H), 5.09 and 5.11 (1H and 1H, each s, =CH₂), 5.89 and 6.36 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS m/z (relative intensity) 442 (M^+ , 100), 424 (47), 406 (15), 339 (34), 287 (27), 271 (42), 269 (36), 251 (26); exact mass calcd for $C_{29}H_{46}O_3$ 442.3447, found
10 442.3442.

20(*S*)-1 α ,25-Dihydroxy-26,27-dimethylene-2 α - and 20(*S*)-1 α ,25-Dihydroxy-26,27-dimethylene-2 β -methyl-19-norvitamin D₃ (48 and 49).

Tris(triphenylphosphine)rhodium (I) chloride (2.3 mg, 2.5 μ mol) was added to dry
15 benzene (2.5 mL) presaturated with hydrogen. The mixture was stirred at room temperature until a homogeneous solution was formed (ca. 45 min). A solution of vitamin 47 (1.0 mg, 2.3 μ mol) in dry benzene (0.5 mL) was then added and the reaction was allowed to proceed under a continuous stream of hydrogen for 3 h. A new portion of the catalyst (2.3 mg, 2.5 μ mol) was added and hydrogen was passed for additional 2 h.
20 Benzene was removed under vacuum, the residue was redissolved in hexane/ethyl acetate (1:1, 2 mL) and applied on Waters silica Sep-Pak. A mixture of 2-methyl vitamins was eluted with the same solvent system (20 mL). The compounds were further purified by HPLC (6.2 mm x 25 cm Zorbax-Sil column, 4 mL/min) using hexane/2-propanol (9:1) solvent system. The mixture (ca. 1:1) of 2-methyl-19-norvitamins 48 and 49 (0.23 mg,
25 23%) gave a single peak at R_V 23 mL. Separation of both epimers was achieved by reversed-phase HPLC (6.2-mm x 25-cm Zorbax-ODS column, 2 mL/min) using

methanol/water (85:15) solvent system. 2 β -Methyl vitamin 49 was collected at R_V 19 mL and its 2 α -epimer 48 at R_V 24 mL.

- 48: UV (in EtOH) λ_{max} 242.5, 251.0, 261.5 nm; ^1H NMR (CDCl_3) δ 0.534 (3H, s, 18-H₃), 0.853 (3H, d, J = 6.6 Hz, 21-H₃), 1.134 (3H, d, J = 6.8 Hz, 2 α -CH₃), 2.13 (1H, ~t, J ~ 12 Hz, 4 β -H), 2.22 (1H, br d, J = 13 Hz, 10 β -H), 2.60 (1H, dd, J = 12.8, 4.6 Hz, 4 α -H), 2.80 (2H, m, 9 β - and 10 α -H), 3.61 (1H, m, w/2 = 23 Hz, 3 α -H), 3.96 (1H, m, w/2 = 11 Hz, 1 β -H), 5.82 and 6.37 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS m/z (relative intensity) 444 (M^+ , 84), 426 (53), 289 (36), 271 (58), 253 (19); exact mass calcd for C₂₉H₄₈O₃ 444.3603, found 444.3602.
- 49: UV (in EtOH) λ_{max} 242.5, 251.0, 261.5 nm; ^1H NMR (CDCl_3) δ 0.547 (3H, s, 18-H₃), 0.859 (3H, d, J = 6.8 Hz, 21-H₃), 1.143 (3H, d, J = 6.8 Hz, 2 β -CH₃), 2.34 (1H, dd, J = 13.7, 3.3 Hz, 4 β -H), 2.43 (1H, br d, J = 13.7 Hz, 4 α -H), 2.80 (1H, br d, J = 12 Hz, 9 β -H), 3.08 (1H, dd, J = 12.9, 4.4 Hz, 10 β -H), 3.50 (1H, m, w/2 = 25 Hz, 1 β -H), 3.90 (1H, m, w/2 = 12 Hz, 3 α -H), 5.87 and 6.26 (1H and 1H, each d, J = 11.3 Hz, 7- and 6-H); MS m/z (relative intensity) 444 (M^+ , 75), 426 (59), 289 (34), 271 (59), 253 (18); exact mass calcd for C₂₉H₄₈O₃ 444.3603, found 444.3611.

Biological Activity of 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-2 α -methyl-19-norvitamin D₃ and 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-2 β -methyl-19-norvitamin D₃ (48 and 49).

Competitive binding of the analogs to the porcine intestinal receptor was carried out by the method described by Dame et al (Biochemistry 25, 4523-4534, 1986).

The differentiation of HL-60 promyelocytic into monocytes was determined as described by Ostrem et al (J. Biol. Chem. 262, 14164-14171, 1987).

Table 8. VDR Binding Properties^a and HL-60 Differentiating Activities^b of 2-Substituted Analogs of 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-19-norvitamin D₃

5	Compound	Compd. no.	VDR Binding ED ₅₀ Binding (M) ratio	HL-60 ED ₅₀ (M)	Differentiation Activity ratio
10	1 α ,25-(OH) ₂ D ₃		8.7 x 10 ⁻¹⁰	1	4.0 x 10 ⁻⁹ 1
	2 α -methyl-26,27-dimethylene- 19-nor-20(S)-1 α ,25-(OH) ₂ D ₃	48	3.5 x 10 ⁻⁹	4.0	4.4 x 10 ⁻¹¹ 0.01
	2 β -methyl-26,27-dimethylene- 19-nor-20(S)-1 α ,25-(OH) ₂ D ₃	49	2.3 x 10 ⁻⁹	2.6	3.2 x 10 ⁻¹⁰ 0.08

^aCompetitive binding of 1 α ,25-(OH)₂D₃ and the synthesized vitamin D analogs to the porcine intestinal vitamin D receptor. The experiments were carried out in triplicate on two different occasions. The ED₅₀ values are derived from dose-response curves and represent the analog concentration required for 50% displacement of the radiolabeled 1 α ,25-(OH)₂D₃ from the receptor protein. Binding ratio is the ratio of the analog average ED₅₀ to the ED₅₀ for 1 α ,25-(OH)₂D₃.

^bInduction of differentiation of HL-60 promyelocytes to monocytes by 1 α ,25-(OH)₂D₃ and the synthesized vitamin D analogs. Differentiation state was determined by measuring the percentage of cells reducing nitro blue tetrazolium (NBT). The experiment was repeated three times. The values ED₅₀ are derived from dose-response curves and represent the analog concentration capable of inducing 50% maturation. Differentiation activity ratio is the ratio of the analog average ED₅₀ to the ED₅₀ for 1 α ,25-(OH)₂D₃.

Table 9. Support of Intestinal Calcium Transport and Bone Calcium Mobilization by 2-Substituted Analogs of 20(S)-1 α ,25-Dihydroxy-26,27-dimethylene-19-norvitamin D₃ in Vitamin D-Deficient Rats on a Low-Calcium Diet^a

Compound	Compd. no.	Amount (pmol)	Ca Transport S/M (mean \pm SEM)	Serum Ca (mean \pm SEM)
none (control)	0		2.7 \pm 0.3 ^b	4.7 \pm 0.2 ^b
1 α ,25-(OH) ₂ D ₃		260	7.2 \pm 0.6 ^c	5.6 \pm 0.2 ^c
2 α -methyl-26,27-dimethylene-	48	32	7.9 \pm 1.0 ^{d1}	6.9 \pm 0.5 ^{d1}
19-nor-20(S)-1 α ,25-(OH) ₂ D ₃		65	9.0 \pm 1.0 ^{d2}	9.0 \pm 0.3 ^{d2}
none (control)	0		3.6 \pm 0.4 ^b	5.0 \pm 0.1 ^b
1 α ,25-(OH) ₂ D ₃		260	5.0 \pm 0.4 ^c	6.3 \pm 0.2 ^c
2 β -methyl-26,27-dimethylene-	49	65	4.9 \pm 0.6 ^{d1}	5.8 \pm 0.2 ^{d1}
19-nor-20(S)-1 α ,25-(OH) ₂ D ₃		260	5.4 \pm 0.7 ^{d2}	9.5 \pm 0.1 ^{d2}

^aWeanling male rats were maintained on a 0.47% Ca diet for 1 week and then switched to a low-calcium diet containing 0.02% Ca for an additional 3 weeks. During the last week, they were dosed daily with the appropriate vitamin D compound for 7 consecutive days. All doses were administered intraperitoneally in 0.1 ml propylene glycol/ethanol (95:5). Controls received the vehicle. Determinations were made 24 h after the last dose. There were at least 6 rats per group. Statistical analysis was done by Student's *t*-test. Statistical data: serosal/mucosal (S/M), panel 1, *b* from *c*, *d*¹, and *d*², *p* < 0.001; panel 2, *b* from *c*, *d*¹, and *d*², *p* < 0.05; serum calcium, panel 1, *b* from *c*, *d*¹, *p* < 0.05, *b* from *d*², *p* < 0.001; panel 2, *b* from *c* and *d*¹, *p* < 0.01, *b* from *d*², *p* < 0.001.

For treatment purposes, the novel compounds of this invention defined by formula I may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-

acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compounds may be administered orally, topically, parenterally, sublingually, intranasally, or transdermally. The compounds are advantageously administered by injection or by intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. Doses of from 0.1 μ g to 50 μ g per day of the compounds are appropriate for treatment purposes, such doses being adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Since the new compounds exhibit specificity of action, each may be suitably administered alone, or together with graded doses of another active vitamin D compound -- e.g. 1 α -hydroxyvitamin D₂ or D₃, or 1 α ,25-dihydroxyvitamin D₃ -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

Compositions for use in the above-mentioned treatment of psoriasis and other malignancies comprise an effective amount of one or more 2-substituted-19-nor-vitamin D compound as defined by the above formula I as the active ingredient, and a suitable carrier. An effective amount of such compounds for use in accordance with this invention is from about 0.01 μ g to about 100 μ g per gm of composition, and may be administered topically, transdermally, orally, sublingually, intranasally, or parenterally in dosages of from about 0.1 μ g/day to about 100 μ g/day.

The compounds may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable solvent or oils, and such preparations may contain in addition other pharmaceutically innocuous or beneficial components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

The compounds are advantageously administered in amounts sufficient to effect the differentiation of promyelocytes to normal macrophages. Dosages as described above are suitable, it being understood that the amounts given are to be adjusted in accordance

with the severity of the disease, and the condition and response of the subject as is well understood in the art.

The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other
5 therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges; each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the
10 form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema.

15 Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions
20 such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

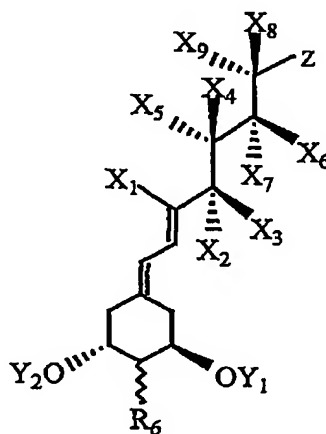
For asthma treatment, inhalation of powder, self-propelling or spray formulations, dispensed with a spray can, a nebulizer or an atomizer can be used. The formulations, when dispensed, preferably have a particle size in the range of 10 to 100 μ .

25 The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or
30 carriers.

In its broadest application, the present invention relates to any 19-nor-2-alkyl analogs of vitamin D which have the vitamin D nucleus. By vitamin D nucleus, it is

meant a central part consisting of a substituted chain of five carbon atoms which correspond to positions 8, 14, 13, 17 and 20 of vitamin D, and at the ends of which are connected at position 20 a structural moiety representing any of the typical side chains known for vitamin D type compounds (such as R as previously defined herein), and at position 8 the 5,7-diene moiety connected to the A-ring of an active 1 α -hydroxy vitamin D analog (as illustrated by formula I herein). Thus, various known modifications to the six-membered C-ring and the five-membered D-ring typically present in vitamin D, such as the lack of one or the other or both, are also embraced by the present invention.

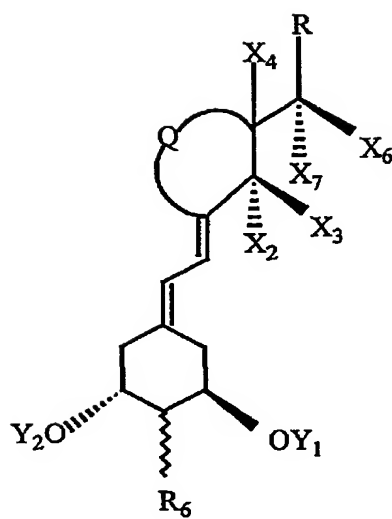
Accordingly, compounds of the following formulae Ia, are along with those of formula I, also encompassed by the present invention:



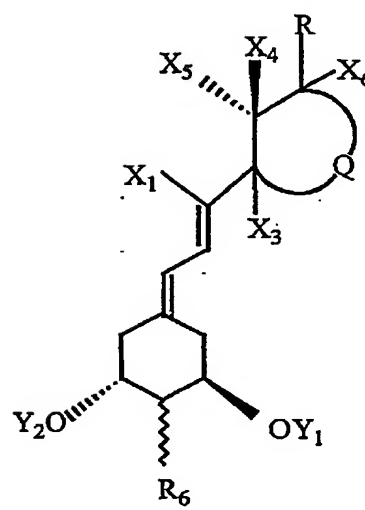
Ia

In the above formula Ia, the definitions of Y₁, Y₂, R₆, and Z are as previously set forth herein. With respect to X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈ and X₉, these substituents may be the same or different and are selected from hydrogen or lower alkyl, i.e. a C₁₋₅ alkyl such as methyl, ethyl or n-propyl. In addition, paired substituents X₁ and X₄ or X₅, X₂ or X₃ and X₆ or X₇, X₄ or X₅ and X₈ or X₉, when taken together with the three adjacent carbon atoms of the central part of the compound, which correspond to positions 8, 14, 13 or 14, 13, 17 or 13, 17, 20 respectively, can be the same or different and form a saturated or unsaturated, substituted or unsubstituted, carbocyclic 3, 4, 5, 6 or 7 membered ring.

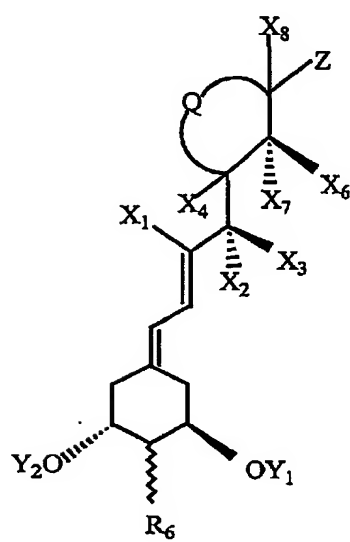
Preferred compounds of the present invention may be represented by one of the following formulae:



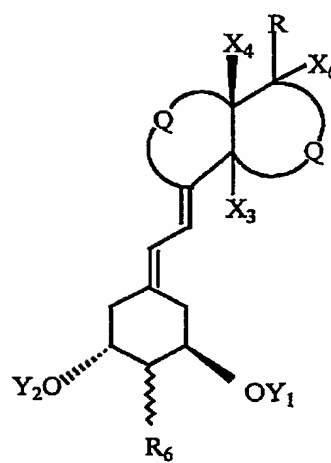
Ib



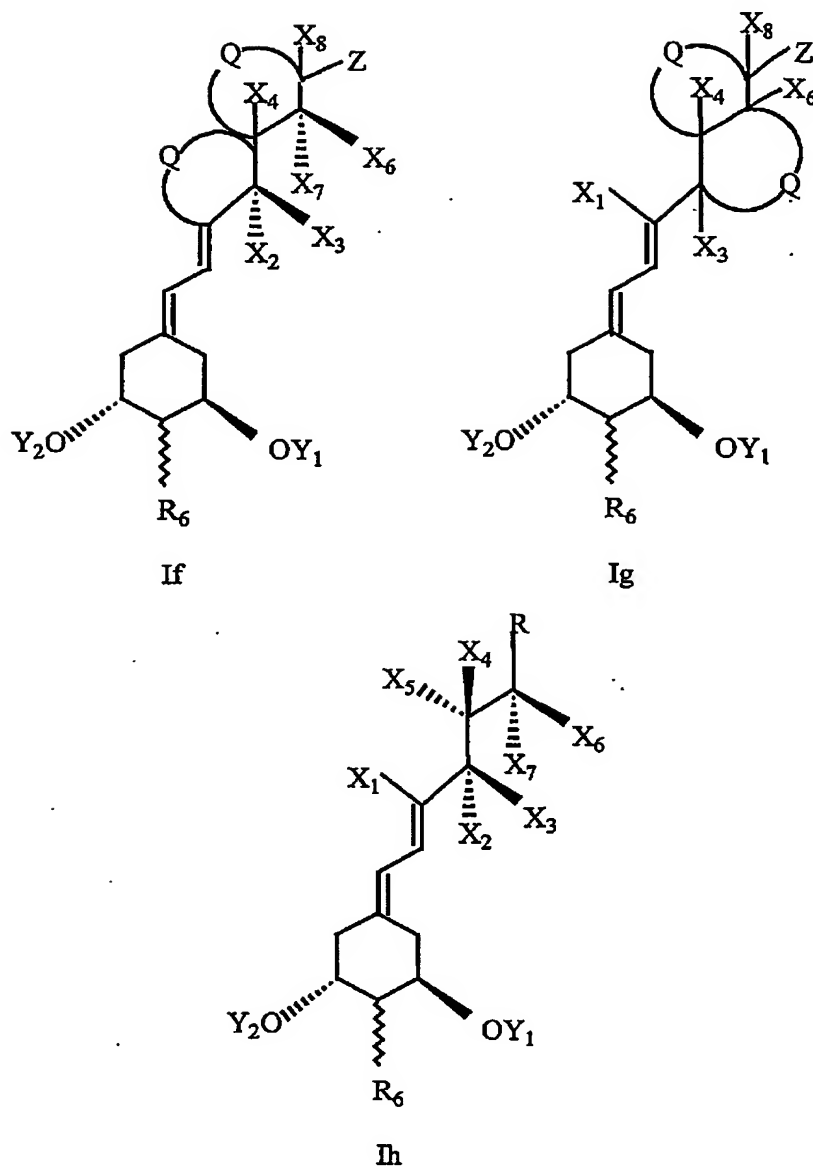
Ic



Id



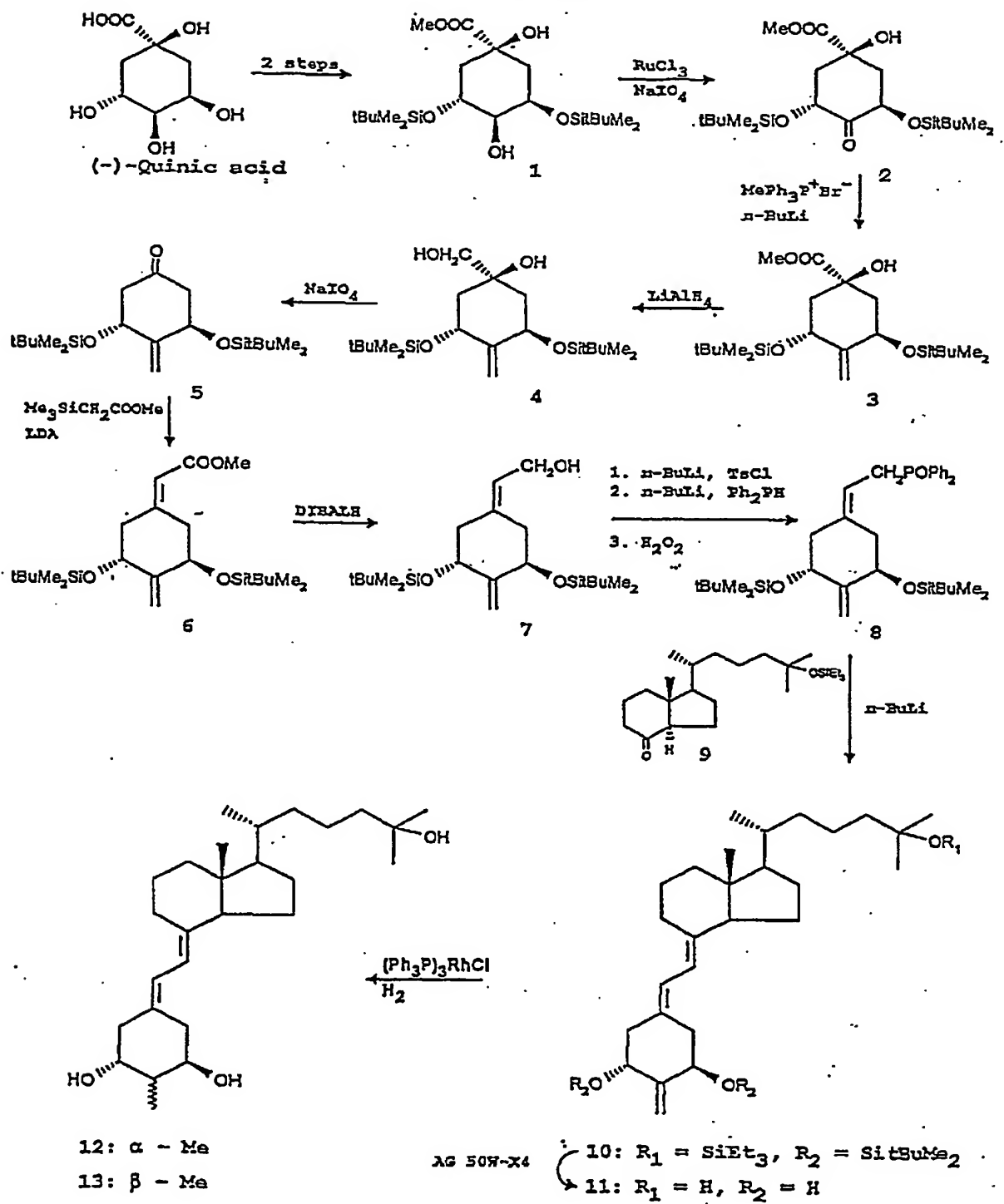
Ie



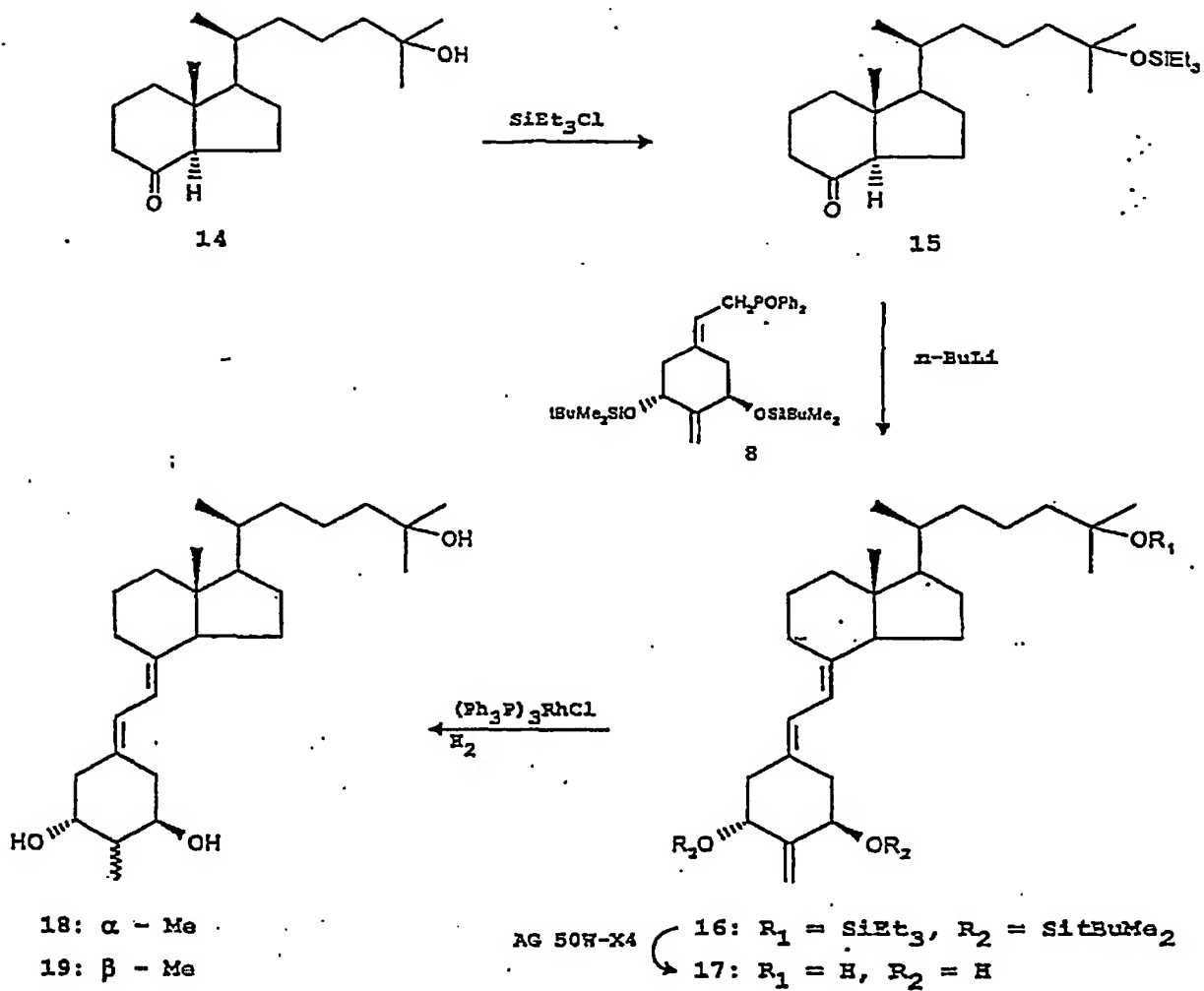
- In the above formulae Ib, Ic, Id, Ie, If, Ig and Ih, the definitions of Y_1 , Y_2 , R_6 , R , Z , X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 and X_8 are as previously set forth herein. The substituent Q represents a saturated or unsaturated, substituted or unsubstituted, hydrocarbon chain comprised of 0, 1, 2, 3 or 4 carbon atoms, but is preferably the group $-(CH_2)_k-$ where k is an integer equal to 2 or 3.

Methods for making compounds of formulae Ia-Ih are known. Specifically, reference is made to International Application Number PCT/EP94/02294 filed 7 July 1994 and published 19 January 1995 under International Publication Number WO95/01960.

SCHEME I

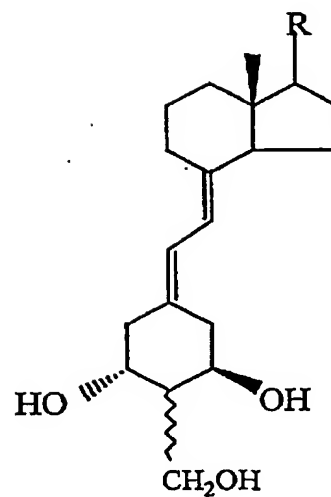


SCHEME II

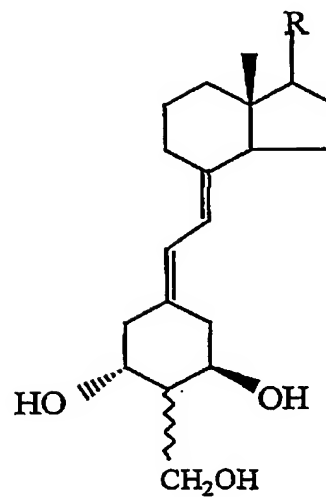


SCHEME III

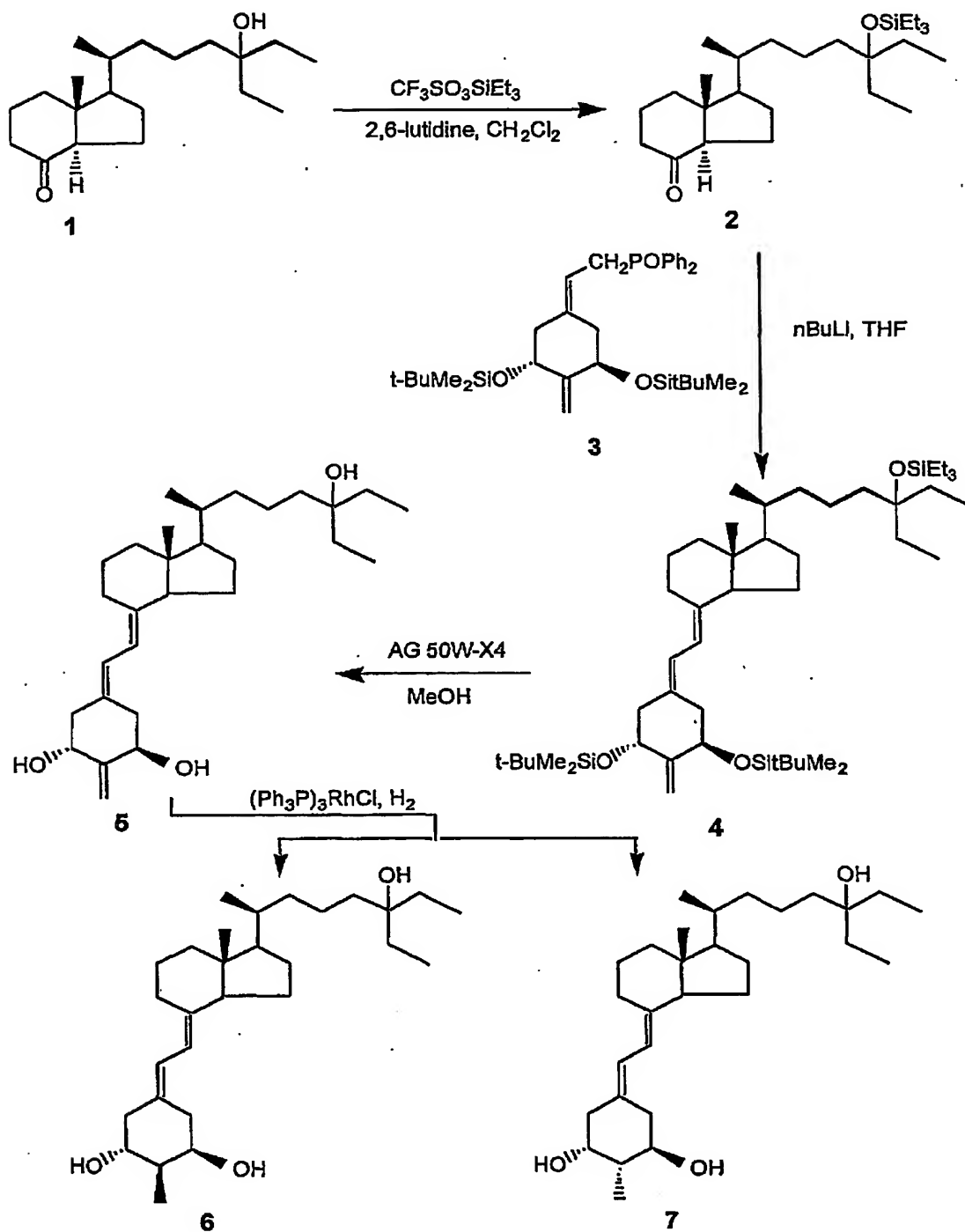
11: 20R



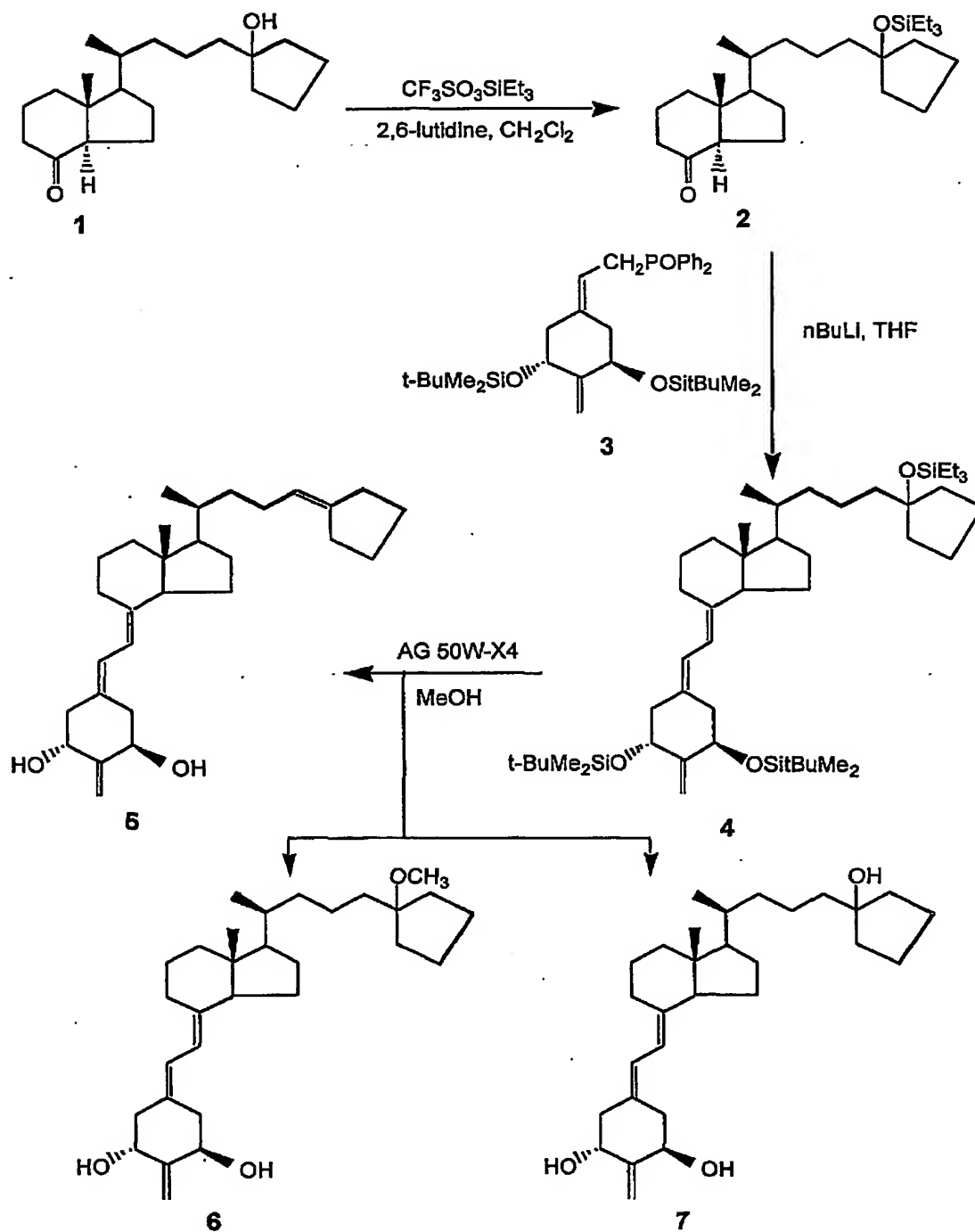
17: 20S



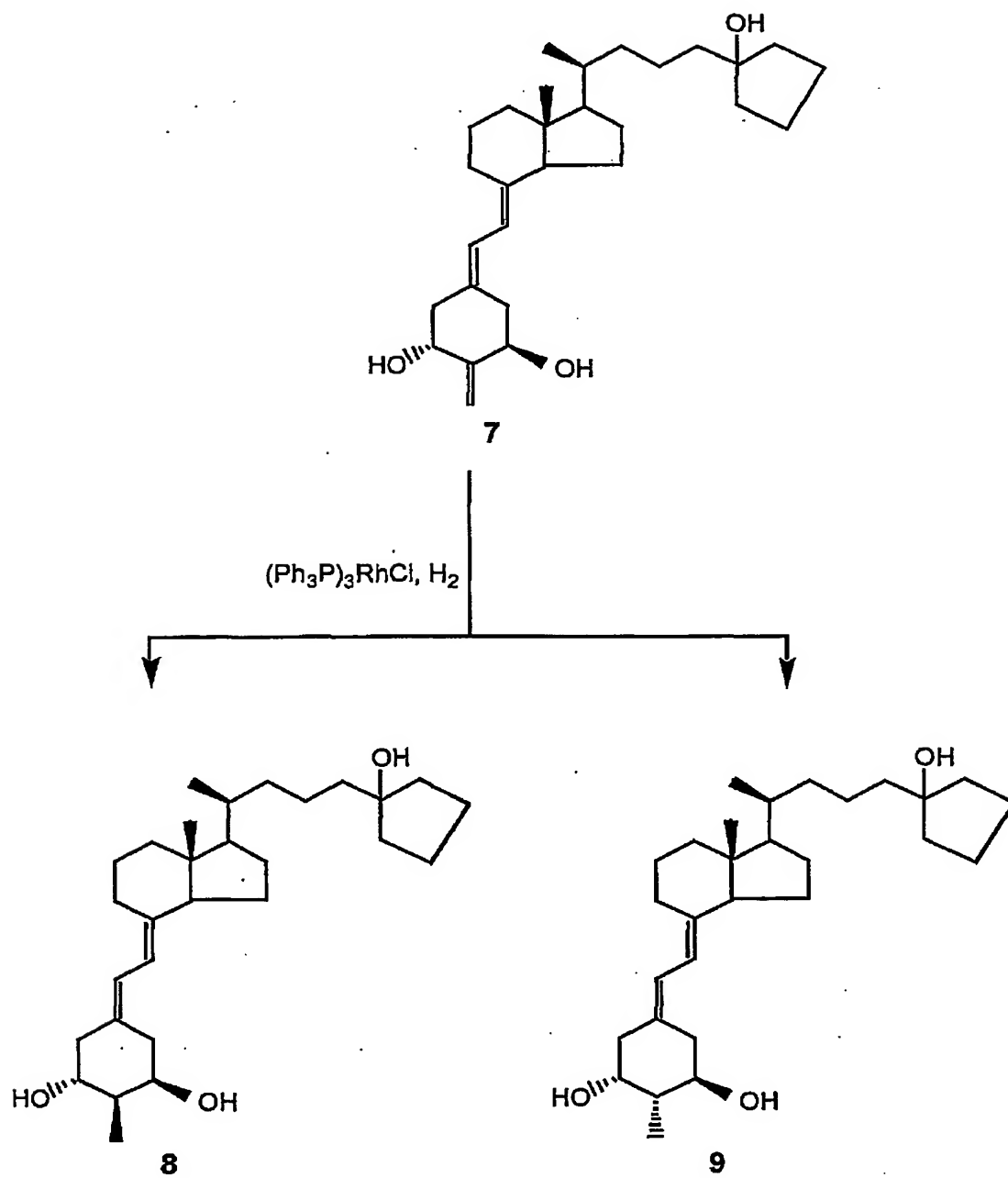
SCHEME IV



SCHEME V



SCHEME VI



CLAIMS

I claim:

1. 19-nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.
2. 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.
3. 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.
4. 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.
5. A pharmaceutical composition containing at least one compound selected from the group consisting of 19-nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃, and 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃, together with a pharmaceutically acceptable excipient.
6. The pharmaceutical composition of claim 5 containing 19-nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃ in an amount from about 0.1 μ g to about 50 μ g.
7. The pharmaceutical composition of claim 5 containing 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃ in an amount from about 0.1 μ g to about 50 μ g.
8. The pharmaceutical composition of claim 5 containing 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃ in an amount from about 0.1 μ g to about 50 μ g.
9. The pharmaceutical composition of claim 5 containing 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃ in an amount from about 0.1 μ g to about 50 μ g.
10. A method of treating metabolic bone disease where it is desired to maintain or increase bone mass comprising administering to a patient with said disease an effective amount of a compound selected from the group consisting of 19-nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃, and 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

11. The method of claim 10 where the disease is senile osteoporosis.
12. The method of claim 10 where the disease is postmenopausal osteoporosis.
13. The method of claim 10 where the disease is steroid-induced osteoporosis.
14. The method of claim 10 where the disease is low bone turnover
osteoporosis.
15. The method of claim 10 where the disease is osteomalacia.
16. The method of claim 10 where the disease is renal osteodystrophy.
17. The method of claim 10 wherein the compound is administered orally.
18. The method of claim 10 wherein the compound is administered
parenterally.
19. The method of claim 10 wherein the compound is administered
transdermally.
20. The method of claim 10 wherein the compound is administered in a dosage
of from 0.1 μ g to 50 μ g per day.
21. The method of claim 10 wherein the compound is 19-nor-26,27-dihomo-
20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.
22. The method of claim 10 wherein the compound is 19-nor-26,27-dihomo-
20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.
23. The method of claim 10 wherein the compound is 19-nor-26,27-
dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.
24. The method of claim 10 wherein the compound is 19-nor-26,27-
dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.
25. A method of treating psoriasis comprising administering to a patient with
said disease an effective amount of a compound selected from the group consisting of 19-
nor-26,27-dihomo-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dihomo-
20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃, 19-nor-26,27-dimethylene-20(S)-2 α -
5 methyl-1 α ,25-dihydroxyvitamin D₃, and 19-nor-26,27-dimethylene-20(S)-2 β -methyl-
1 α ,25-dihydroxyvitamin D₃.
26. The method of claim 25 wherein the compound is 19-nor-26,27-dihomo-
20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.

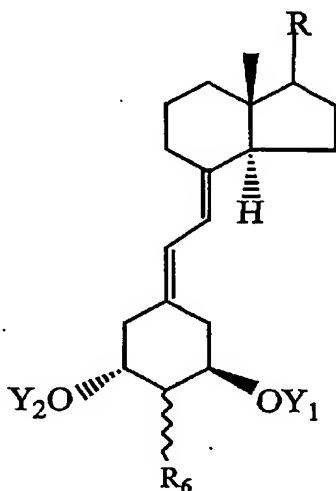
27. The method of claim 25 wherein the compound is 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

28. The method of claim 25 wherein the compound is 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.

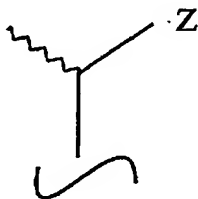
29. The method of claim 25 wherein the compound is 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

30. The method of claim 25 wherein said effective amount comprises about 0.01 μ g/day to about 100 μ g/day of said compound.

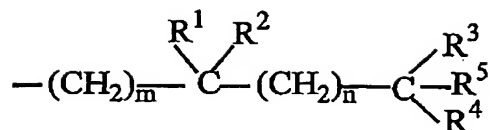
31. A method of treating a cancerous disease comprising administering to a patient with said disease an effective amount of a compound having the formula:



where Y₁ and Y₂, which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, R₆ is selected from alkyl, hydroxyalkyl and fluoroalkyl, and where the group R is represented by the structure:



where the stereochemical center at carbon 20 may have the R or S configuration, and where Z is selected from Y, -OY, -CH₂OY, -C \equiv CY, -CH=CHY, and -CH₂CH₂CH=CR³R⁴, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, -COR⁵ and a radical of the structure:



- 15 where m and n, independently, represent the integers from 0 to 5, where R¹ is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C₁₋₅-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R², R³, and R⁴, independently, is selected from deuterium, deuterioalkyl, hydrogen, fluoro, trifluoromethyl and C₁₋₅ alkyl,
- 20 which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R¹ and R², taken together, represent an oxo group, or an alkylidene group, =CR²R³, or the group -(CH₂)_p-, where p is an integer from 2 to 5, and where R³ and R⁴, taken together, represent an oxo group, or the group -(CH₂)_q-, where q is an integer from 2 to 5, and where R⁵ represents hydrogen, hydroxy, protected hydroxy,
- 25 C₁₋₅ alkyl or -OR⁷ where R⁷ represents C₁₋₅ alkyl, and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups -CH(CH₃)-, -CH(R³)-, or -CH(R²)- at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

32. The method of claim 31 where the disease is leukemia.
33. The method of claim 31 where the disease is colon cancer.
34. The method of claim 31 where the disease is breast cancer.
35. The method of claim 31 where the disease is prostate cancer.
36. The method of claim 31 wherein the compound is administered orally.
37. The method of claim 31 wherein the compound is administered parenterally.
38. The method of claim 31 wherein the compound is administered transdermally.
39. The method of claim 31 wherein the compound is administered in a dosage of from 0.1μg to 50μg per day.
40. The method of claim 31 wherein the compound is 19-nor-26,27-dihomo-20(S)-2α-methyl-1α,25-dihydroxyvitamin D₃.

41. The method of claim 31 wherein the compound is 19-nor-26,27-dihomo-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

42. The method of claim 31 wherein the compound is 19-nor-26,27-dimethylene-20(S)-2 α -methyl-1 α ,25-dihydroxyvitamin D₃.

43. The method of claim 31 wherein the compound is 19-nor-26,27-dimethylene-20(S)-2 β -methyl-1 α ,25-dihydroxyvitamin D₃.

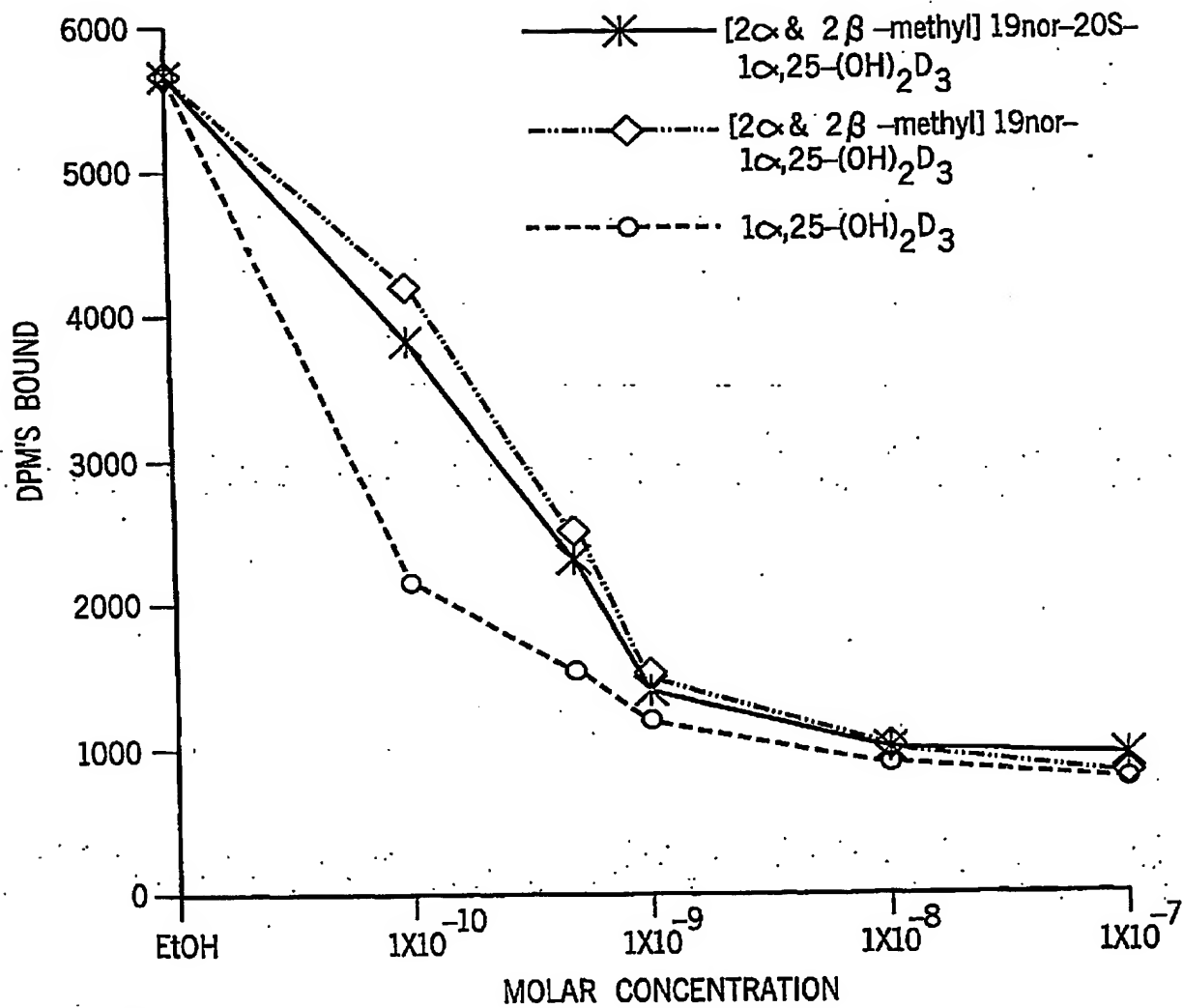


FIG. 1

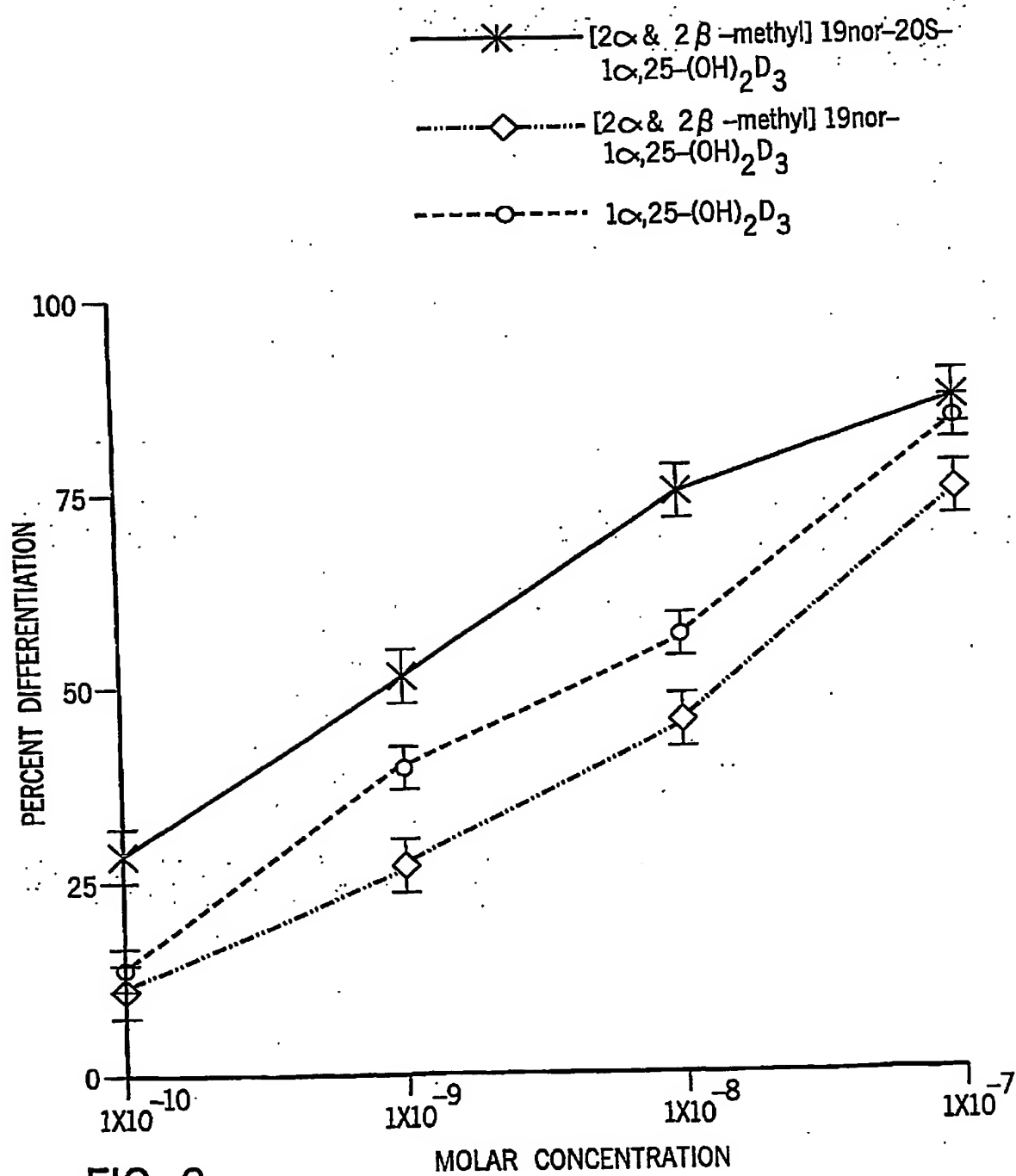


FIG. 2

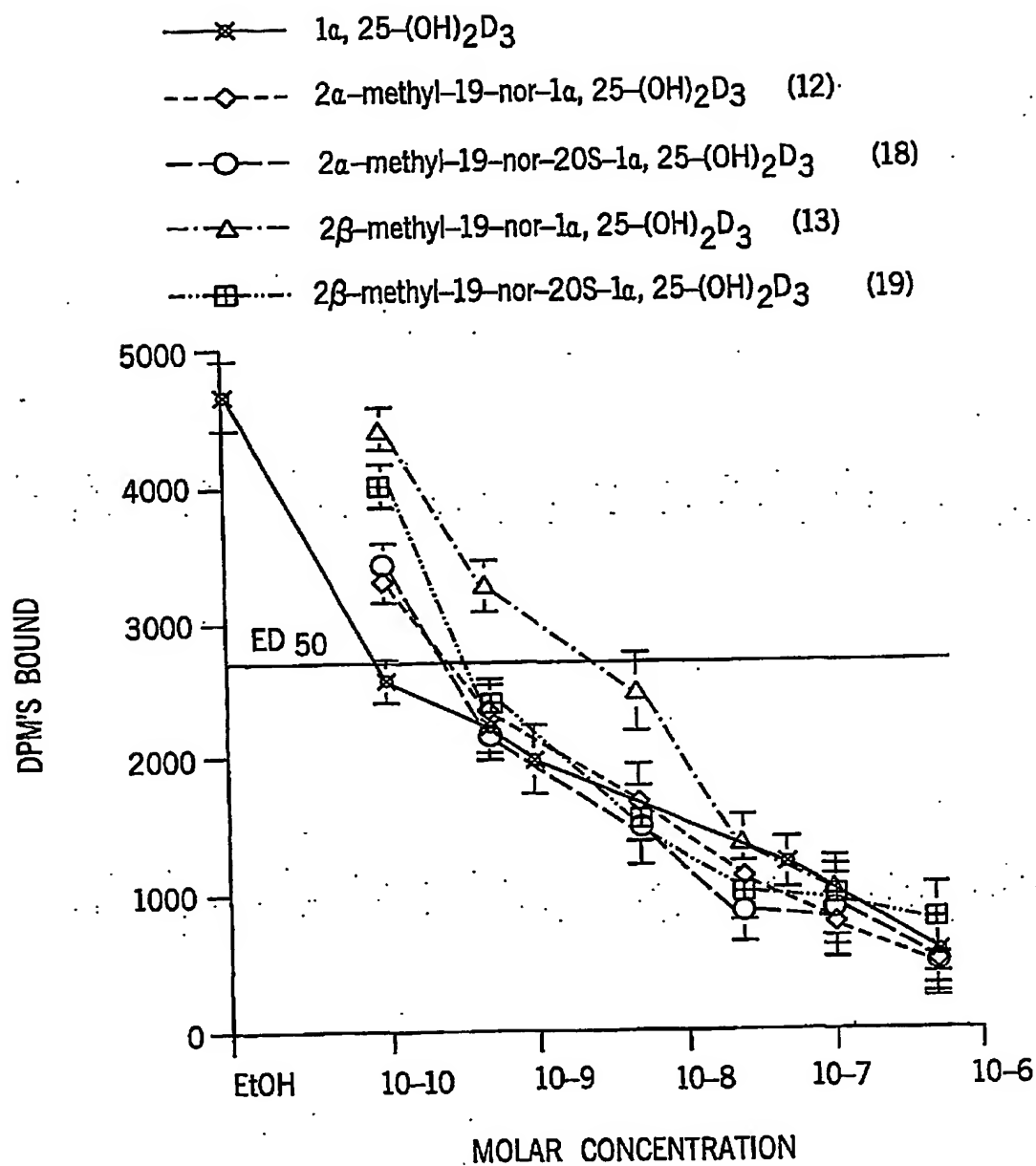


FIG. 3

- ×— 1 α , 25-(OH) $_2$ D $_3$
 ---◇--- 2 α -methyl-19-nor-1 α , 25-(OH) $_2$ D $_3$ (12)
 ---○--- 2 α -methyl-19-nor-20S-1 α , 25-(OH) $_2$ D $_3$ (18)
 ---△--- 2 β -methyl-19-nor-1 α , 25-(OH) $_2$ D $_3$ (13)
 ---■--- 2 β -methyl-19-nor-20S-1 α , 25-(OH) $_2$ D $_3$ (19)

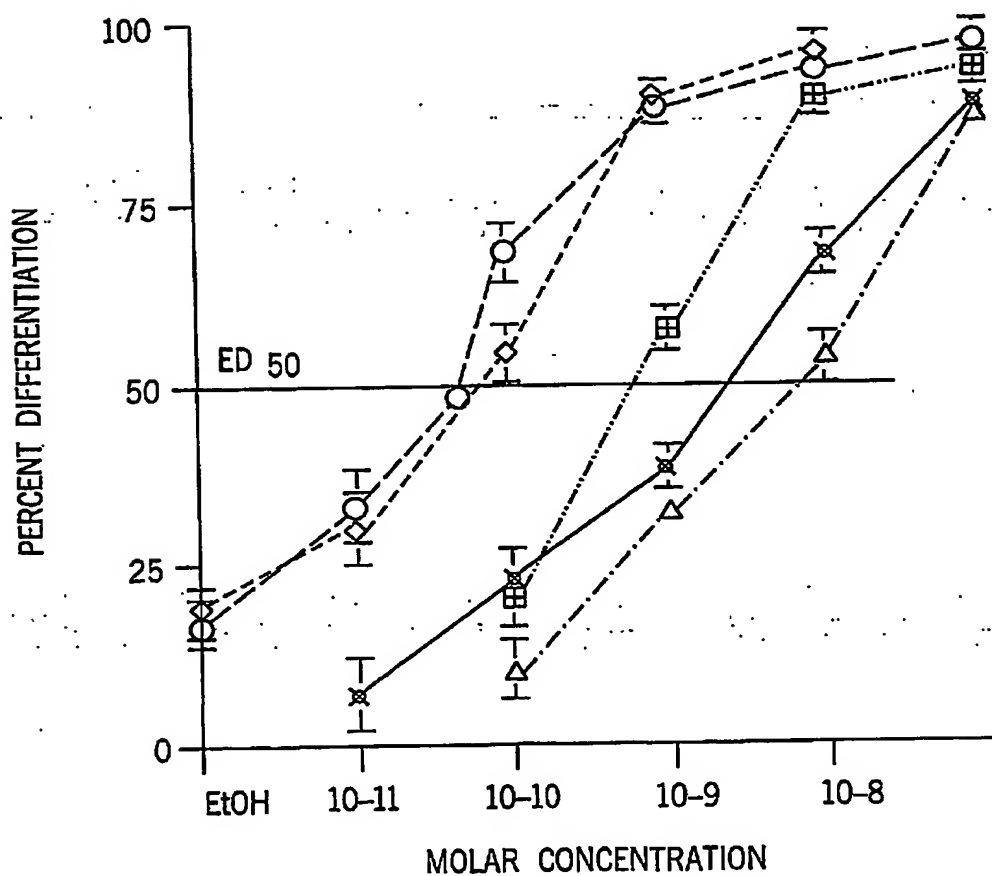


FIG. 4

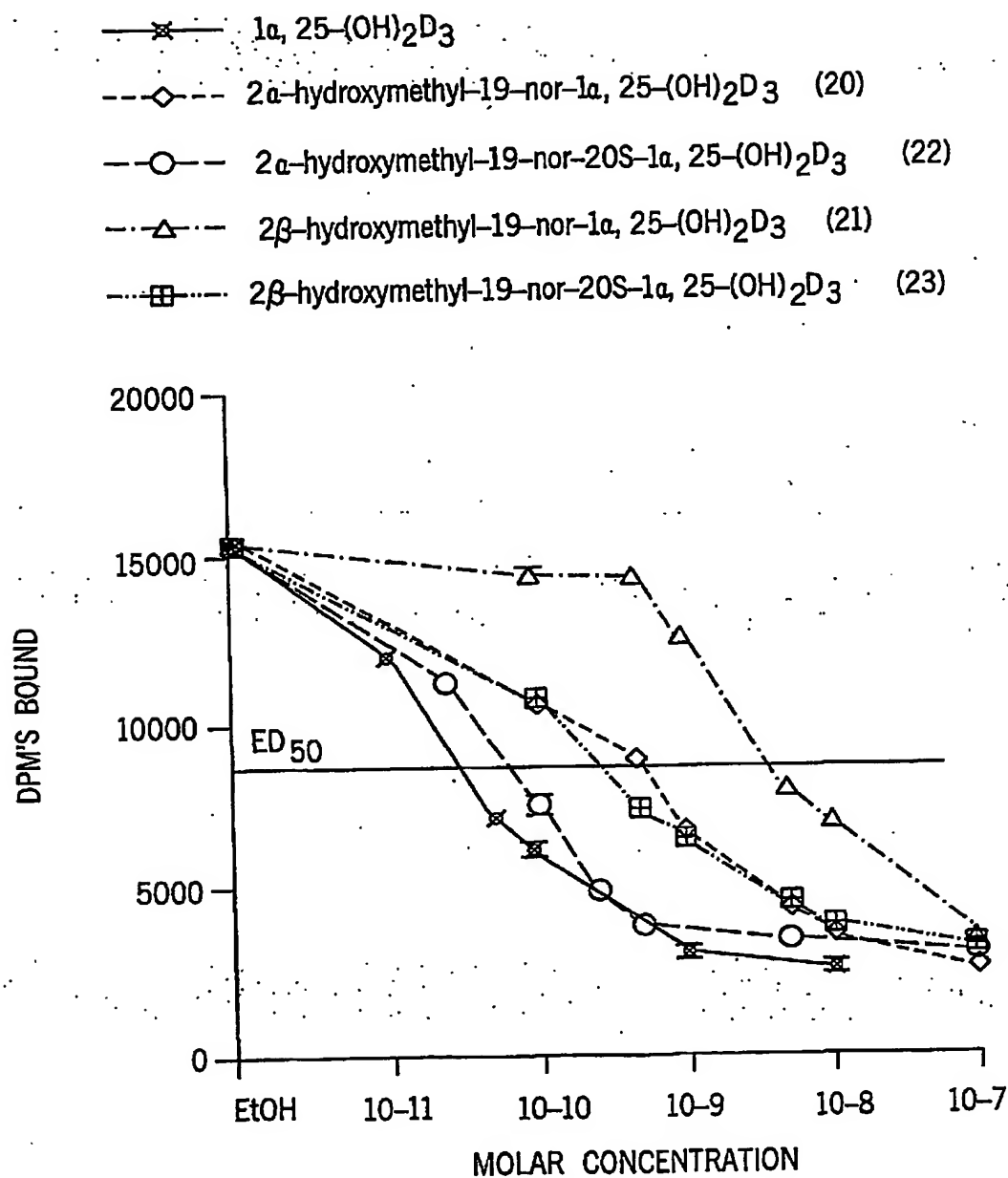


FIG. 5

- x— $1\alpha, 25-(OH)_2D_3$
- ◇--- 2α -hydroxymethyl-19-nor- $1\alpha, 25-(OH)_2D_3$ (20)
- 2α -hydroxymethyl-19-nor-20S- $1\alpha, 25-(OH)_2D_3$ (22)
- △--- 2β -hydroxymethyl-19-nor- $1\alpha, 25-(OH)_2D_3$ (21)
- 2β -hydroxymethyl-19-nor-20S- $1\alpha, 25-(OH)_2D_3$ (23)

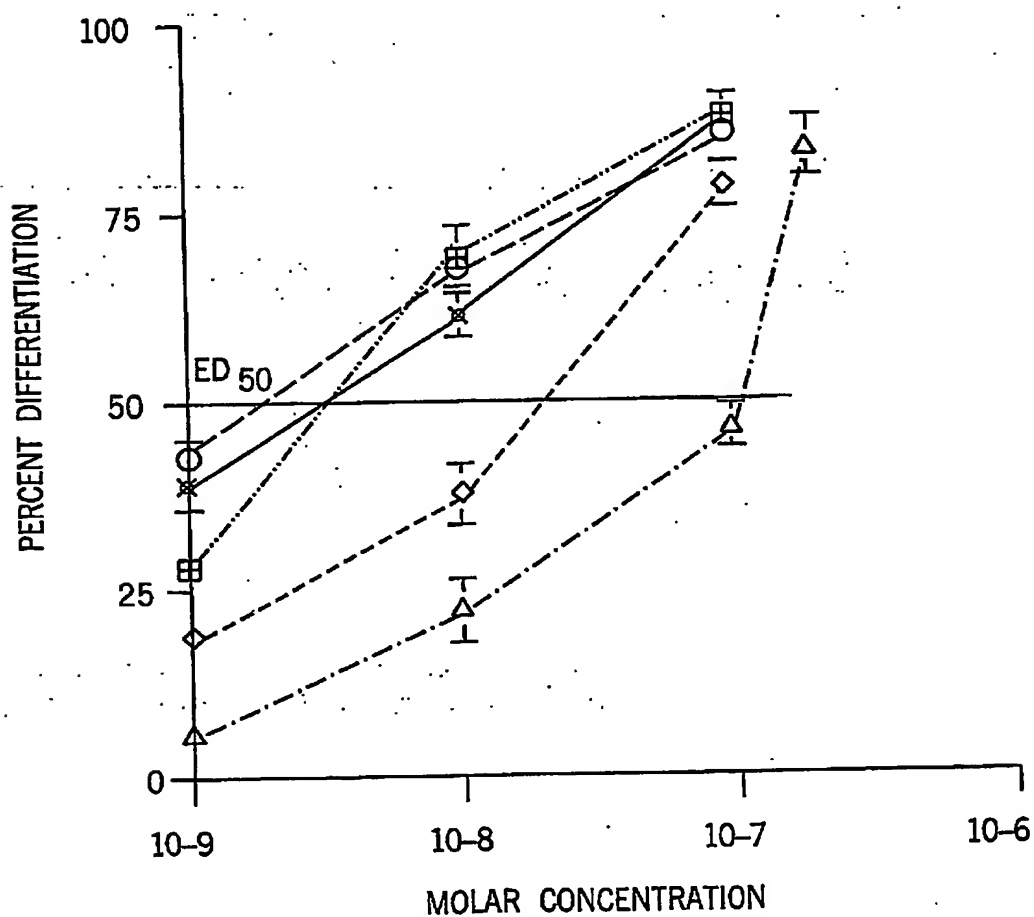


FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/10094

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07C401/00 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 41500 A (WISCONSIN ALUMNI RESEARCH FOUNDATION) 24 September 1998 (1998-09-24) page 3, line 11 - page 6, line 19; page 7, lines 24-28; page 34, lines 14-23	31-39
A	claims	1-30
X	R.R. SICINSKI ET AL: J. MED. CHEM., vol. 41, no. 23, 1998, pages 4662-4674, XP002106465 pages 4667-4669, biological evaluation	31
X	K. MIKAMI ET AL: SYNLETT, no. 12, 1999, pages 1899-1902, XP001002275 page 1899, scheme 1, compound 2	31, 32
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

20 July 2001

Date of mailing of the international search report

19/09/2001

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/10094

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 10548 A (WINCONSIN ALUMNI RESEARCH FOUNDATION) 2 March 2000 (2000-03-02) the whole document	1-24, 31-43
A	WO 90 00541 A (WISCONSIN ALUMNI RESEARCH FOUNDATION) 25 January 1990 (1990-01-25) page 21, line 14 - page 22, line 14; claims	3-5, 8-11, 16-18, 23, 24, 31, 32, 36, 37, 39, 42, 43

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Information on patent family members

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